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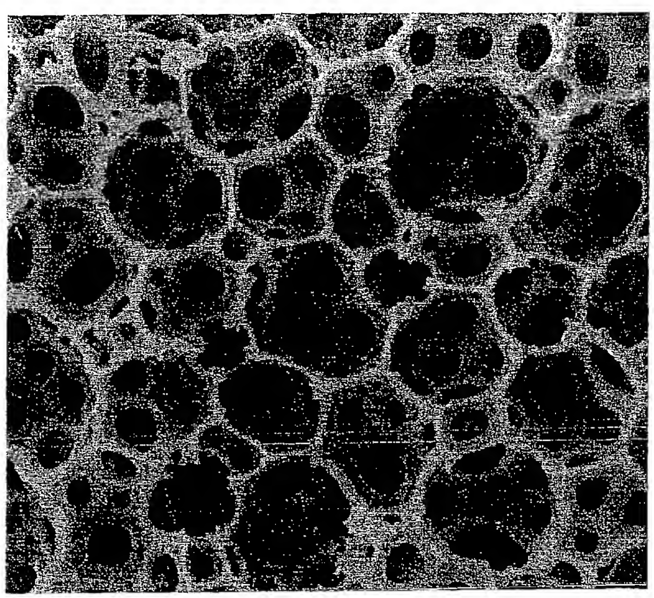
(54) Title: MICROPOROUS POLYMERS



(57) Abstract: The present invention relates to a process for the preparation of composite microporous polyHIPE polymers, to numerous uses of microporous polyHIPE polymers and to their conversion to functional materials.

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MICROPOROUS POLYMERS

The present invention relates to a process for the preparation of composite microporous polyHIPE polymers, to numerous uses of microporous polyHIPE polymers and to their conversion to functional materials.

A well-established process for preparing microporous polyHIPE polymers is described in WO-A-00/34454. In the first instance, an aqueous solution of a polymerisation initiator (usually potassium persulphate for example) is dosed into an oil phase containing (as the main components) a styrene monomer and a cross-linking agent (divinyl benzene) together with a surfactant (Span 80) with mixing in a batch mixer to form an emulsion. Subsequent mixing results in the reduction of the pore size. After formation of the water-in-oil high internal phase emulsion in which the volume of the dispersed aqueous phase may be as high as 97-98%, the temperature is increased to 60°C and polymerisation is initiated. After completion of polymerisation and cross-linking, the internal phase is evaporated and the polymer is dried at 100°C. If the pore size is very small, the emulsion is more stable and even though pore coalescence takes place during polymerisation, it is insufficient to cause total phase separation and the formation of a polymer with significantly reduced phase volume. The pores of the polymers obtained through this route are classified as type-2 pores. Such coalescence pores are very large and (for example) interaction of an enzyme with the pores will be prohibited. If no coalescence takes place, basic type-1 pores are obtained. The structure of type-1 pores with large or very small interconnecting holes are shown in Figures 1a and 1b respectively.

The microcellular polyHIPE polymers disclosed in WO-A-00/34454 exhibit controlled pore sizes with oil phase fillers, water soluble polymers or surface coatings such as minerals (*eg* hydroxyapatite) polyethylene oxide, polyethylene glycol or sodium carboxymethylcellulose or electrolyte or particulate matter. Moreover small amounts of an inclusion (organic or inorganic) may be placed in the aqueous solution or the continuous phase and polymerisation may be achieved without destabilisation. However, depending on the concentration and physical-chemical nature of the inclusion, the emulsion can rapidly destabilise and separate out thereby failing to form a microporous structure.

It has been found that in seeking to prepare composite polymers by the route described in WO-00/34454, high concentrations of an inclusion cause the emulsion to destabilises before polymerisation is complete. Phase separation during polymerisation is also encountered even if there are no macromolecular or particulate inclusions present. If the density of the discontinuous phase is very high compared with the continuous phase, phase separation may still take place. This situation may be encountered when large amounts of electrolyte are dissolved in the discontinuous (*eg* aqueous) phase meaning that the resulting mineral deposition is suitable for surface coating only.

WO-A-00/34454 also discloses the preparation of animal cell support systems with controlled pore and interconnecting hole size (both of which effect cell proliferation, cell differentiation and cell penetration into the pores of the support material). Moreover, WO-A-00/34454 discloses a method of opening microscopic channels into the microcellular polymeric support system in order to feed or stimulate cell growth and cell function.

The application of process intensification (PI) to biotechnology is one of the major challenges of bioreaction engineering. PI in biotechnology has certain inherent restrictions in terms of "*intensification fields*" such as temperature, pressure, concentration of reactants/products, detrimental effects of contaminants, mechanical stresses, deformation rates and electric fields. Due to these restrictions on the type of PI-driving forces, Bioprocess Intensification (BI) can be achieved in the first instance through the reduction of the diffusion path for the reactants and products and through the creation of an optimally suitable environment for the biocatalysts and microorganisms. Consequently, biocatalyst membranes and specially designed bioreactors (such as jet loop reactors) are available to intensify biochemical reactions (Giorno and Drioli, 2000, TIBTECH. 18:339-349; and Bayhan *et al.*, Water Research. 3, 2001: 2191-2200). Supported biocatalysts are often employed in order to enhance catalytic activity and stability and to protect enzymes/micro-organisms from mechanical degradation and deactivation (Giorno *supra*; and De Bartolo *et al.*, J. Materials Sci-Materials in Medicine. 12, 2001: 959-963).

Immobilization of cells is one technique to improve productivity of bioreactors.

Immobilized cells are cells that are entrapped within or associated with an insoluble matrix. Various methods are used for immobilization including covalent coupling, adsorption, entrapment in a three-dimensional scaffold, confinement in a liquid-liquid emulsion and entrapment within a semi permeable membrane. Bioreactors with immobilized cells and enzymes have several advantages over bioreactors with free cells and free enzymes. Systems with immobilized cells permit the operation of bioreactors at flow rates that are independent of the growth rate of the microorganisms employed. Catalytic stability can be greater for immobilized cells than for free cells. Some immobilized microorganisms tolerate higher concentrations of toxic compounds than free cells, when the cell support media acts as a sink for the excess toxin. However in the current biocatalyst support technology, the presence of the support itself introduces mass transfer restrictions for the substrate/product/ nutrient diffusion to and from the biocatalyst. These disadvantages are also valid when supports are used to grow animal cells *in vitro*. As shown in WO-A-00/34454, the efficiency of cell function (for example production of collagen II) is dependent on the microarchitecture (*ie* pore and interconnect size) and surface chemistry of the support.

Enzymes are widely used in catalysis and it is generally desirable for them to be reusable (particularly if they are expensive). For this purpose, the enzyme generally needs to be immobilised. However, immobilisation may reduce the bioactivity and reaction rate due to changes in chain configuration and the presence of additional diffusion barriers. Enzyme immobilisation with no restriction on chain mobility is desirable. The removal of the products and the supply of fresh substrate are achieved by diffusion.

In conventional ion exchange resin beads, the pores are very similar and very small. Although the surface area is large, when such materials are exposed to polluted streams, the inner pores are generally not utilised due to the formation of a fouling layer on the surface which prevents the penetration of the substrate into the inner pores. In other words, although the active sites for absorption on the surface are occupied immediately, the penetration of the pollutants (metal ions and nutrients for example) is hindered. This is also important in bacterial support applications of microporous polymers where bio-fouling hinders the diffusion of substrate if the pores are small.

Highly porous metallic structures are widely utilised including *inter alia* as lightweight structural engineering materials, in catalytic reactions, as catalytic converters, as conductive and/or high temperature membranes/electrodes, in fuel cell applications, as miniaturised reactors and heat exchangers. Such microporous metallic structures are generally prepared by sintering.

The use of a microporous polymer to act as a template and allow the deposition on the surface of a metal from a solution has been in general conducted without success. However some very weak metallic porous particles have been obtained (Sotiropoulos *et al*, Material. Lett., 35 (1998) 383). In this method, it is necessary to activate the polymer surface with a metal such as palladium.

Soil has a porous structure and pores contain water which may be polluted. Soil typically has a negative surface charge. To balance this charge, cations such as sodium, calcium and magnesium as well as other heavy metal ions line up along the walls of the pores. In most cases, counter-anions in soil water are chloride, sulphate, and nitrate, as well as other ionic anions. When a DC electric field is applied by inserting two electrodes into the soil containing water, the cations on the surface of the soil start sliding towards the cathode by electric attraction. This boundary layer movement creates a bulk water movement towards the cathode. Within the bulk soil water, the individual cations also move towards the cathode while the anions move towards the anode. Water moving towards the cathode carrying the metal ions also carry the organic toxins. At the anode, oxygen gas evolves while the cathode reaction results in hydrogen gas evolution. Most importantly, water is transported under the electric field from anode to cathode.

The system described in WO-A-00/34454 may also be applied to bacteria when they are used in re-mediation. Bacteria are initially seeded into the microcellular supports and allowed to proliferate and subsequently be used for re-mediation. In most applications, (especially in *in situ* re-mediation), the substrate composition will be varied and it will contain toxins which will be detrimental to bacterial survival. Most of the co-toxins present in soil and water will be heavy metals in addition to organic toxins targeted by bio

re-mediation. In the presence of multi-toxins, bacterial re-mediation processes should incorporate a protection system for the bacteria.

The present invention seeks to improve the preparation of composite microporous/microcellular polymers to achieve high concentration of inclusions within the pores of the emulsion or at the phase interface without destabilising the emulsion. More particularly the present invention relates to a process for preparing by agitation (*eg* deformation or shaking) of the high internal phase emulsion a composite polyHIPE polymer having a high concentration of inclusions.

Thus viewed from a first aspect the present invention provides a process for preparing a composite polyHIPE polymer comprising:
adding together a first phase and a second phase, wherein the first phase and the second phase are immiscible and either the first phase or the second phase is polymerisable;
adding to one or more of the first phase, the second phase or the high internal phase emulsion one or more inclusions;
causing the formation of a high internal phase emulsion;
causing the onset of polymerisation of the high internal phase emulsion; and
agitating the high internal phase emulsion at least after the onset of polymerisation.

By way of example, where the inclusion is an enzyme, the concentration of encapsulated enzyme may be a factor of 10 greater than that which is achievable by conventional processes.

The process of the invention leads to a composite polyHIPE polymer which is microporous and microcellular and in which the inclusions are (with or without bonding) entrapped, incorporated, intercalated, inserted or adsorbed in the micropores of the microporous composite polyHIPE polymer (*ie* other than exclusively on the surface). The term "*inclusion*" is intended to relate exclusively to exogenous species (*ie* not species indigenous to (*eg* dispersed or dissolved in) the first phase, the second phase or the high internal phase emulsion or indigenous to the successful carrying out of steps (c) and (d)).

The term "*agitating*" is intended to include shaking or deforming (but not exclusively mixing).

The term "*polymerisation*" herein is intended to include one or more of linear or branched polymerisation, linear or branched copolymerisation and cross-linking (or combinations thereof).

Preferably the first phase is continuous and the second phase is dispersed. The continuous phase may be a non-aqueous phase. The dispersed phase may be an aqueous phase. Preferably the continuous (non-aqueous) phase is an oil phase and further comprises a surfactant (*eg* Span 80).

The first or second phase which is polymerisable (the polymerisable phase) may be or contain one or more of the group consisting of high or low molecular weight, natural or synthetic monomers, co-monomers, oligomers, co-oligomers, macromonomers, polymers, co-polymers and mixtures thereof. The term monomer/co-monomer is intended to cover single monomer/co-monomer units or a block of repeating monomer/co-monomer units (such as a dimer or trimer).

By way of example, the composite polyHIPE polymer may be a polyvinyl, polyaryl, polyheterocycle (*eg* polyheteroaryl), oligoaryl (*eg* oligoheteroaryl) or oligoheterocycle-based composite polyHIPE polymer.

The polymerisable phase may contain an optionally ring substituted heterocyclic moiety (such as a 5- or 6-membered optionally ring substituted heterocyclic moiety). The polymerisable phase may contain an optionally ring substituted heteroaromatic moiety. The optionally ring substituted heterocyclic moiety may contain one, two or three heterocyclic atoms which may be the same or different. Preferably the (or each) heterocyclic atom is selected from the group consisting of nitrogen, sulphur, oxygen and phosphorous, preferably the group consisting of nitrogen, oxygen and sulphur, particularly preferably the group consisting of nitrogen and sulphur. By way of example, the optionally ring substituted heterocyclic moiety may be selected from the group consisting of optionally

ring substituted thiophene, furan, pyridine, imidazole, isothiazole, isooxazole, pyran, pyrazine, pyridazine, pyrazole, pyridine, pyrimidine, triazole, oxadiazole, pyrrole, indazole, indole, indolizine, pyrrolizine, quinazoline and quinoline.

The polymerisable phase preferably contains an optionally ring substituted carboaromatic moiety (or aryl moiety). For example, the moiety may be selected from the group consisting of an optionally ring substituted phenyl, benzyl, anthracenyl, phenanthrenyl and napthalenyl moiety. Preferably the moiety is an optionally ring substituted phenyl moiety (*eg* an *o*- or *p*-substituted phenyl moiety). The phenyl moiety may be ring substituted (*eg o*- or *p*-substituted) with a group which enhances its electronic properties (preferably a group which has an electron donating effect). A particularly preferred optionally ring substituted phenyl moiety is an optionally ring substituted styryl moiety (*eg* an *o*- or *p*-substituted styryl moiety).

In a preferred embodiment, the polymerisable phase comprises a styrene monomer or a styrene co-monomer and optionally (but preferably) a cross-linking agent. A preferred styrene co-monomer is a styrene/alkyl alkylacrylate co-monomer (wherein each alkyl group is independently selected from the group consisting of a linear, branched or cyclic C_{1-6}-alkyl group such as methyl, ethyl, propyl, isopropyl, butyl isobutyl, pentyl or hexyl). Particularly preferred is a styrene/ethyl hexylacrylate co-monomer which provides an advantageously flexible hydrocarbon chain (see EP-A-0239360).

The cross-linking agent may be selected from the group consisting of proteins, silicates, monomers, co-monomers, oligomers, co-oligomers, macromonomers, polymers and co-polymers. Specific examples include divinyl benzene, ethylene diacrylate, N-N'-diallyltartardiamine, N-N'(1,2 dihydroxyethane)-bis-acrylamide and N-N'-N"-triallyl citrictriamide. Preferred is divinylbenzene. In order to resist polymer swelling, high concentrations of cross-linking agent may be used (*eg* 25% DVB instead of 8%).

A polymerisable phase containing amine moieties or cross-linking agents are useful in the covalent immobilisation of enzymes.

The polymerisable phase may contain a moiety which is hydrophilic. Preferably the polymerisable phase contains polar functional moieties, particularly preferably NO_2 and $S(O)_2OH$.

Preferably the polyHIPE polymer is a phosphated, nitrated or (preferably) sulphonated polyHIPE polymer or has attached ligands.

Polymerisation may be carried out thermally and/or chemically. For example, the temperature of the emulsion may be elevated to a polymerisation temperature. Typically, the polymerisation temperature is 60°C or more.

For the purposes of carrying out step (d) chemically, the first (*eg* non-aqueous) phase or the second (*eg* aqueous) phase may contain a polymerisation initiator. For example, an aqueous phase may include sodium or (preferably) potassium persulphate or a non-aqueous phase may contain azobisisobutyronitrile or 1,1-azobis(cyclohexanecarbonitrile).

Step (e) may be followed by additional chemical steps such as *inter alia* condensation steps or by homogenisation (*eg* by agitation). Additives may be added to the first (*eg* non-aqueous) phase (for example to achieve coalescence if desired).

The composite polyHIPE polymer may take any convenient form such as a particulate, powder, fibre, monolithic (*eg* disc) or membrane form.

For the avoidance of doubt, step (b) may be carried out before, during or after step (c).

Step (e) may be carried out manually (*eg* by manual shaking) or mechanically (*eg* using a mechanical vibrator or agitator to effect mechanical shaking).

Step (e) may be carried out intermittently or continuously.

Step (e) may be carried out substantially vertically, substantially horizontally, substantially circularly or in an orbital motion (or any combination or sequence of these). For example,

agitation by shaking may be carried out (for example) in a cell culture shaker in which the frequency and/or amplitude of shaking may be controlled together with the temperature of the emulsion. The microstructure of the composite polyHIPE polymer may be dependent on the frequency, amplitude and total shaking time of the emulsion.

Step (e) may be carried out at least until shortly after the onset of polymerisation (*eg* at least during the initial stages of polymerisation). Provided in this instance that the emulsion does not contain a large concentration of inclusions, the emulsion will generally be stable. If the concentration of inclusions is very high, there is an increased tendency for the emulsion to separate out during polymerisation. In this instance, agitation is preferably carried out at least until gelation.

The inclusion (*eg* functional inclusion) may be an organic or inorganic species within the pores or on (or as an integral part of) the walls of the pores of the microporous composite polyHIPE polymer. The microporous composite polyHIPE polymers may be used as functional materials in their own right or they may be modified in embodiments of the process of the invention to obtain microporous structures with a specific function.

Suitable inclusions include *inter alia* small particles (such as molecular particles or microscopic particles *eg* colloidal or submicron particles or nanoparticles), enzymes, bacteria, bacterial spores (*eg* in the micropores where they may activated *in situ* at the point of use), fibres (*eg* metal, carbon, polymeric or natural fibres), metal species and cells (*eg* animal or plant cells). Preferred inclusions will be discussed in detail below.

(1) Small Particles

Where the inclusion is a small particle (*eg* a colloidal or submicron particle or nanoparticle), it is preferred that step (e) is carried out at least until gelation. By this embodiment, relatively large amounts of small particles (*eg* 20wt% by volume of the polymer) may be incorporated into the walls of the microporous composite polyHIPE polymer. Specific examples of submicron particles or nanoparticles are sintering agents, metal species (*eg* elemental metal), carbon black, colloidal silica, carbon, glass or

combinations thereof.

It has been found that microporous composite polyHIPE polymers containing small particles are mechanically much stronger than the parent (*ie* non-composite) microporous polyHIPE polymer.

A preferred colloidal or submicron particle or nanoparticle (*eg* elemental metal or a sintering agent) is coated with a silane coupling agent and is added in step (b) to the emulsion after step (c) (*eg* shortly before gelation), wherein the first phase is a continuous phase (*eg* an oil phase). The silane coupling renders the metal species or sintering agent hydrophobic and prevents partial breakdown of the emulsion (especially near the metal surface) as a result of coalescence.

Suitable silane coupling agents are generally compatible with the polymerisable phase. For example, styryl amine functional silanes are preferred. Preferably step (e) is carried out at least until gelation.

In a preferred embodiment of the process, a submicron particle or nanoparticle (*eg* carbon black or silica) is added in step (b) to the second phase during or after step (c), wherein the second phase is an aqueous phase and the first phase is a continuous phase (*eg* an oil phase). Preferably during step (e) the temperature is increased. Step (e) is typically carried out by shaking (*eg* in an incubator shaker) at about 120rpm. Preferably step (e) is carried out at least until gelation.

The composite polyHIPE polymers of this embodiment may be used to covalently immobilize enzymes. When colloidal silica is used for example as the inclusion, it may be treated with a suitable silane coupling agent (such as (gamma-aminopropyl)triethoxysilane) which transforms the surface of the silica to have amino functionality suitable for immobilisation of enzymes.

In a preferred embodiment, the composite polyHIPE polymer is hydrophilic. Particularly preferably the composite polyHIPE polymer is sulphonated. This may be carried out by

soaking the composite polyHIPE polymer in sulphuric acid or (preferably) by adding to the second (aqueous) phase dilute sulphuric acid. These approaches are discussed in detail in a PCT patent application filed on an even date herewith claiming priority from GB0215833.5. The hydrophilic (*eg* sulphonated) polyHIPE polymers and processes generically and specifically described therein are incorporated herein by reference.

When conventional polyHIPE polymers are rendered hydrophilic (*eg* sulphonated), they swell upon contact with water. However, the hydrophilic composite polyHIPE polymers prepared in accordance with this embodiment of the process of the invention are dimensionally stable (*ie* do not swell) in water. They are therefore useful as ion exchange material and in rotating disk reactors.

In a preferred embodiment of the process, a precursor to a small particle (preferably an electrolyte) is added in step (b) to the second phase before step (c) and the process further comprises:
(f) converting the precursor to a small particle into a small particle.

The second phase is preferably aqueous and in step (b) the precursor (*eg* electrolyte) dissolves therein, wherein step (f) is carried out by precipitation (*eg* using a base). A high concentration of the precursor may be used to yield a high concentration of small particles of precipitate (*eg* mineral). Preferably step (e) is carried out at least until gelation.

By this embodiment, small particles of minerals (*eg* magnesium, iron and calcium salts) may be incorporated in the pores of a microporous composite polyHIPE polymer. These composite polyHIPE polymers may provide superior cell or bacteria adhesion. A preferred mineral is hydroxyapatite.

<u>(2) Fibres</u>

Carbon, high strength fibres such as Kevlar, hydrophilic or metallic fibres are preferred inclusions. Where the inclusion is such a fibre, the composite polyHIPE polymer may have added strength. Moreover in some instances, the composite polyHIPE polymer may

advantageously be used to obtain a polyHIPE polymer with a gradient of pores or a network of microchannels or capillaries. Microporous polyHIPE polymers with a gradient of pore size are advantageous in the sense that active sites are more accessible for the binding of ions or organic toxins (*ie* blinding of pores is prevented). The presence of a network of large capillaries (about 100 microns) ensures that substrates or metal ions can reach active sites in the polyHIPE polymer faster.

In a preferred embodiment of the process, carbon, metal (*eg* copper) or hydrophilic fibres are added in step (b) to the emulsion after step (c) (*eg* shortly before gelation). Step (e) is typically carried out by shaking (*eg* in an incubator shaker) at 60-300rpm (*eg* about 120rpm) at elevated temperature (*eg* about 60°C). Preferably step (e) is carried out at least until gelation.

The resulting composite polyHIPE polymer is itself mechanically strong and therefore useful. However preferably the process further comprises: (f') removing the fibres from the composite polyHIPE polymer. For example, step (f') may be carried out mechanically (*eg* by pulling out the fibres) or chemically (*eg* by dissolving the fibres).

For example, step (b) may be carried out in a specially constructed mould in which a 3D network of fibres are constructed (either in 1, 2 or 3 directions) to which the high internal phase emulsion is added in step (b). If desired, a second inclusion may have been (or may be) added to the first phase, second phase or high internal phase emulsion. By this embodiment, a network of capillaries may be created within the bulk of the polyHIPE polymer. The bulk polyHIPE polymer may be granulated to a desired size to obtain powdered microporous polyHIPE polymer with a network of capillaries or cut into suitable size pieces as packing materials. Such packing materials may also be useful in other separation processes such as distillation and liquid extraction. If the fibres used in obtaining the capillary network are hydrophilic, the capillary pore wall interface will have an open, interconnected pore structure.

Alternatively for example, fibres (*eg* short fibres) are in step (b) dispersed into the emulsion and the process further comprises: (f') removing the fibres from the composite

polyHIPE polymer. For example, after polymerisation, the bulk polyHIPE polymer may be granulated and step (f') carried out by dissolving the fibres in a solvent or in an acid to leave a microporous polyHIPE polymer with a gradient of pore size. Preferred in this embodiment are hydrophilic (*eg* sulphonated or nitrated) composite polyHIPE polymers (as disclosed for example in PCT patent application filed on an even date herewith claiming priority from GB0215833.5).

(3) Enzymes

The composite polyHIPE polymers prepared by the present invention may host high concentrations of enzymes. There appears to be substantially no inhibition of enzyme activity despite the polymerisation reaction. In contrast to conventional methods for encapsulating enzymes, the enzymes are substantially free and the composite polyHIPE polymers may be used as flow through enzymatic bioreactors and/or to protect the enzyme.

Where the inclusion is an enzyme, the composite polyHIPE polymer may be used in soil remediation (*eg* as a non-ionic barrier to organic toxins) or in biotechnology.

In a preferred embodiment, the enzyme is added in step (b) to the second phase which is a dispersed (*eg* aqueous phase). The second phase may additionally contain co-enzyme or enzyme stabiliser.

Alternatively if the enzyme is to be added in step (b) to the first phase which is continuous, the enzyme is preferably dispersed in oil (such as cyclohexane or heptane). The continuous phase may additionally contain resorcinol and formalin together with a suitable surfactant such as hexadecylpropyl sulphobetaine. When the emulsion is formed, the addition of a catalyst such as p-toluenesulphonic acid causes a rigid polymer to be formed within minutes through a polycondensation polymerisation as described by US-A-4985468. This type of encapsulation has the advantage of being carried out at low temperature.

Preferably step (e) is carried out before and after the onset of polymerisation, particularly preferably until gelation.

Some enzymes break down in the presence of strong oxidising agents such as potassium sulphate persulphate used as an aqueous polymerisation initiator. In this case, oil phase soluble initiators should be used.

Preferred enzymes are invertase and urease. They may be encapsulated at amounts of 1g/l or more (*eg* 5-11 g/l).

The process of the invention generally leads to non-chemically bonded (free) enzyme. Chemical (covalent) bonding may be desirable and well known techniques are available to achieve this. It is generally desirable to immobilise the enzymes within the pores of the polyHIPE polymer using relatively low phase volume (*eg* 80 %) of the dispersed phase and ensuring that the pore size of the microporous polyHIPE polymer is also small. Small pore size also results in smaller interconnect size, increased enzyme-support interaction and (during polymerisation) the rate of coalescence is reduced which in turn reduces level of coalescence pores. When small pores (say 1μm or less) are produced, it is also possible to incorporate more enzyme into the pores since the emulsion coalescence during polymerisation will not be fast enough to break down the emulsion completely.

Thus in a preferred embodiment, the process further comprises: functionalising the composite polyHIPE polymer in which the inclusion is an enzyme. Particularly preferably the step of functionalising the composite polyHIPE polymer comprises: immobilizing the enzyme.

The step of functionalising may be carried out chemically or physically (*eg* thermally). For this purpose, the surface of the pores preferably bear functional groups such as amino, carboxyl and hydroxyl groups. For example, the polymerisable phase may contain a moiety (*eg* a monomer optionally with styrene as co-monomer) bearing one or more of these functional groups. The functional group may be activated using known reagents such as cyanogen bromide and glutaraldehyde.

Alternatively, the step of functionalising may be carried out by coating the composite

polyHIPE polymer with a monomer which contains a functional group and allowing the monomer to polymerise on the surface of the composite polyHIPE polymer. Enzyme attachment to the functional group may then be conducted.

Due to very low interconnect size, when enzymes are encapsulated within the pores during emulsification, they are not able to escape because of their size and their hydrophobic interaction with the walls of the pores. However, the size of the enzyme molecules can be increased by crosslinking the enzymes using glutaraldehyde or bis-diazobenzidine and 2,2-disulfonic acid (or other known cross-linking agents).

PolyHIPE polymers with immobilized enzymes can be used in biosensors. When used as biosensors, their response can be expected to be very high due to high enzyme concentration. When enzyme encapsulated/immobilized polymers in monolithic form are used as bioreactors, they can either be employed in stationary form with flow of the substrate solution perpendicular to the monolith or they can be rotated in which case the flow will be both in three dimension, radial, angular and perpendicular direction. Such flows will enhance mass transfer rates.

Viewed from a further aspect the present invention provides a method for enzymatically catalysing a reaction comprising:
(1) preparing a composite polyHIPE polymer in a process as hereinbefore defined wherein the inclusion is an enzyme or obtaining a fibre entrapped enzyme; and
(2) effecting mass transfer of substrates or products by forced convection.

Mass transfer (*eg* reaction product removal and substrate supply to the entrapped enzyme) are intensified in accordance with this aspect of the invention.

In a preferred embodiment, step (1) comprises preparing a composite polyHIPE polymer in a process as hereinbefore defined wherein the inclusion is an enzyme and step (2) comprises: (2) effecting mass transfer either by compressing/decompressing the composite polyHIPE polymer or oscillating the pressure or flow rate in a flow through assembly.

Compressing the highly microporous polyHIPE polymer or oscillating the pressure in a flow through system allows mixing at microscopic level within the pores thereby allowing rapid mass transfer. During the decompression cycle, fresh solution containing a substrate is taken inside the pores and during the compression cycle, solution containing the reaction products is forced out of the pores. It is well known that pressure or flow rate oscillations in mixers which consist of a series of tubes separated by smaller diameter tubes of very short length (baffled plate mixers or multiple expansion contraction static mixers) result in intensified mixing. This aspect of the invention essentially represents a miniaturised version of such mixers.

In an alternative preferred embodiment, step (1) comprises: preparing a composite polyHIPE polymer in a process as hereinbefore defined in the shape of a disc with enzymes immobilised on the surface and/or encapsulated or immobilised in the pores and step (2) is carried out by rotating the disc whilst the substrate solution is fed from the centre of the disc.

This embodiment creates high acceleration and allows substrate solution to migrate radially outwardly. Due to the presence of both radial and angular flow, intense mixing within the pores accelerates mass transfer.

In a further alternative preferred embodiment, step (1) comprises: preparing a composite polyHIPE polymer in a process as hereinbefore defined in the shape of a disc with enzymes immobilised on the surface and/or encapsulated or immobilised in the pores and step (2) is carried out by rotating the disc whilst the substrate solution is fed from the top surface of the disc.

This embodiment constitutes a cross-flow rotating porous disc reactor configuration in which the permeate goes through the porous disc containing the encapsulated/immobilised enzyme while the retantate flows radially outwardly for collection and recycle. This situation is particularly suitable where the substrate solution contains suspended solids or macromolecules dissolved in water. This is mainly encountered in waste water treatment.

In a fibre entrapped enzyme system, step (2) may be carried out by pressing (*eg* periodically pressing) the fibre. This reduces the void volume of fibre bundles forcing the reaction products out from the reaction zone. When the fibre bundle is reinstated (*ie* void volume is increased), the substrate is taken into the reaction zone for reaction.

(4) Bacterial Spores

The inclusions may be bacterial spores. Unlike their parent bacterium, the bacterial spores may be more able to survive the polymerisation temperature and environment.

In a preferred embodiment, bacterial spores are added in step (b) to the emulsion during or after step (c).

After polymerisation (and any other subsequent steps such as washing), bacterial spores may be activated to obtain composite polyHIPE polymers with encapsulated bacteria for use in bio-conversions (as discussed hereinafter).

(5) Ligands

Ligands (such as metal specific ligands) may be incorporated in a polyHIPE polymer in accordance with the process of the invention.

In a preferred embodiment, ligands are added in step (b) to the emulsion before step (c). Particularly preferably the ligand is added to the second phase which is a dispersed phase.

Such ligand encapsulation may impart desirable functional or surface characteristics to the polyHIPE polymer. They may be usefully further modified.

(6) Bacteria

Where the inclusion is bacteria, the composite polyHIPE polymer may be used in soil remediation (*eg* as a non-ionic barrier to organic toxins) or in biotechnology.

In the currently available seeding technique, penetration of microorganisms into the pores of a microporous polymer from its surface is a slow process and requires a cell proliferation front to propagate into the pores. Overall cell proliferation rate is reduced since it is controlled by cell transport (penetration) into the pores. The transport of the cells into the pores is further hindered by the reduction of the interconnecting holes (in size and number) as a result of cell proliferation and extra-cellular matrix formation resulting in biofilm.

From a further patentable viewpoint, the present invention is based on the recognition that bacteria may be forced seeded into the micropores of the microporous structure of a polyHIPE polymer of the invention before cell proliferation (provided that the cells do not undergo mechanical lysis) and allowed to grow and spread to colonise the polyHIPE polymer. For a highly resistant bacteria, it may even be possible to seed the bacteria at the emulsification stage.

Viewed from a further aspect the present provides a method for cultivating bacteria comprising:
forced seeding bacteria into a high internal phase emulsion polymerisable into a polyHIPE polymer or into the micropores of a polyHIPE polymer; and
allowing the bacteria to colonise the polyHIPE polymer.

Forced seeding may be carried out by exerting a force on the bacteria (*eg* the bacterial solution). Generally the step of forced seeding is carried out slowly. For example, force may be exerted on the solution mechanically (*eg* by pumping using for example a piston) or using a compressed gas (*eg* air). Typically the pump rate is in the range 1 to 4 ml/minute (*eg* about 1ml/min).

Preferably the microporous polyHIPE polymer is one which is specifically or generically described in WO-A-00/34454, in a PCT patent application filed on an even date herewith claiming priority from GB0215833.5 or herein.

The method of this aspect of the invention ensures that cell concentration is uniform across the polyHIPE polymer and is not dependent on cell proliferation and subsequent advance of the proliferation front into the support. This serves to accelerate overall processing time which is one of the key aims of Bioprocess Intensification and to ensure a high concentration of bacteria so that reaction efficiency is heightened. Additionally the polymer support serves to protect the bacteria.

Preferably the method further comprises: causing the onset of a bioreaction. In a preferred embodiment, the bioreaction is a flow-through bioreaction (and the system represents a flow through bioreactor), particularly preferably a recirculatory flow-through bioreaction. Operated as a flow-through system in the degradation of phenol in water, the volumetric phenol utilization rate is some 30-400 times more than that reported in the literature. This represents a clear bioprocess intensification.

Preferably the bacteria is allowed to colonise the polyHIPE polymer to cover the surface of the pores in a monolayer.

Bacteria may cover the surface of the pores in a monolayer form when the pore size is relatively small (*eg* 20 microns) and it does not produce any extracellular matrix. When the pores are very large (about 80 microns or more), multilayer coverage and extra-cellular matrix production may occur.

For this aspect of the invention, the polyHIPE polymer preferably adopts a monolithic structure (*eg* a slice).

In a preferred embodiment, the polyHIPE polymer comprises microchannels, particularly preferably (in a flow through bioreactor) microchannels perpendicular to the reaction flow. Efficient oxygen supply can be achieved by using microchannels perpendicular to the direction of flow in the bio-reactor (which is important for aerobic bio-conversions). These channels may be coated with high oxygen transporting polymers such as cross-linked polyorganosiloxanes.

In a preferred embodiment of this aspect of the invention, the polyHIPE polymer has a structure with a hydrophobic core and a hydrophilic (*eg* sulphonated) skin. Preparation of such polymers is described in WO-A-00/34454. For example, a polyHIPE polymer may be soaked in sulphuric acid and heated (*eg* to a temperature in excess of 100°C). A hydrophobic core can be seeded with bacteria whilst a hydrophilic (*eg* sulphonated) skin can act as an absorbent for water and dissolved nutrients.

(7) Animal or Plant Cells

The present invention is further based on the recognition that the above forced seeding technique can be useful to achieve a high concentration of animal or plant cells in microporous polyHIPE polymers in a short culture time (*eg* in tissue engineering). Since each seeded cell has a large surface area for further proliferation, proliferation rate can be expected to increase.

Viewed from a further aspect the present provides a method for cultivating cells comprising:

forced seeding cells into a high internal phase emulsion polymerisable into a polyHIPE polymer or into the micropores of a polyHIPE polymer; and

allowing the cells to colonise the polymer.

Preferably the microporous polyHIPE polymer is one which is specifically or generically described in WO-A-00/34454, in PCT patent application filed on an even date herewith claiming priority from GB0215833.5 or herein.

From a further patentable viewpoint, water containing ionic species and organic toxins (*eg* from soil) may be allowed to come into contact with a bacteria or enzyme containing composite polyHIPE polymer for the degradation of organic toxins.

Viewed from a further aspect the present invention provides use of a composite polyHIPE polymer in bioremediation (*eg* as a non-ionic barrier to organic toxins), wherein the inclusion is bacteria or an enzyme.

Preferably bioremediation is carried out on water from soil.

It is likely that bacterial function will be effected by the support architecture (*ie* pore and interconnect size) as well as the chemistry of the polyHIPE polymer. Therefore, the structural architecture of the polymer and the precise chemistry may need to be optimised for a given bacterial re-mediation.

The bacteria or enzyme support can be in monolithic or particulate form with large capillary channels providing easy access for solution (*eg* water).

The present invention also seeks to improve polymerisation of certain high internal phase emulsions via re-dispersion of the high internal phase emulsion into a more hydrophilic surfactant and agitating the emulsion to prevent the polymer destabilising.

Viewed from a further aspect the present invention provides a process for preparing a polyHIPE polymer comprising:
adding together a continuous phase and an aqueous phase, wherein the continuous phase and the aqueous phase are immiscible and the continuous phase contains a first surfactant and is polymerisable;
causing the formation of a high internal phase emulsion;
redispersing the high internal phase emulsion into a second surfactant, wherein the hydrophilicity of the second surfactant is greater than the hydrophilicity of the first surfactant;
causing the onset of polymerisation of the high internal phase emulsion into a polyHIPE polymer; and
agitating the high internal phase emulsion at least after the onset of polymerisation .

Particle size is dependent on a large number of factors such as the HLB of the second surfactant and its concentration, agitating conditions and the degree of pre-polymerisation.

Agitation may be carried out intermittently or continuously, manually (*eg* by manual

shaking) or mechanically (*eg* using a mechanical vibrator or agitator to effect mechanical shaking). Agitation may be carried out substantially vertically, substantially horizontally, substantially circularly or in an orbital motion (or any combination or sequence of these). For example, agitation by shaking may be carried out (for example) in a cell culture shaker in which the frequency and/or amplitude of shaking may be controlled together with the temperature of the emulsion. The microstructure of the polyHIPE polymer may be dependent on the frequency, amplitude and total shaking time of the emulsion.

Agitation may be carried out at least until shortly after the onset of polymerisation. Agitation is preferably carried out until gelation.

The process of this aspect of the invention is cost-effective in preparing polyHIPE polymers with very large pores extending throughout (like a capillary) which improves the accessibility of pores in subsequent utilities.

The polyHIPE polymer is typically obtained in particulate form.

Preferably the hydrophile/lipophile (HLB) balance of the second surfactant is in the range 8 to 15, particularly preferably 9 to 14, more particularly preferably 10 to 13, especially about 12.

From a further patentable viewpoint, the present invention is based on the recognition that non-catalytic surface reactions can be conducted within the pores or on the surface of a heated microporous polyHIPE polymer by passing a metal solution without the need to pre-condition the polymer.

Viewed from a further aspect the present invention provides a process for metallising a microporous material comprising:

establishing the microporous material at a substantially steady elevated temperature; and

passing metal solution though the microporous material.

By judiciously avoiding heating of the metal solution itself, any tendency of the metal solution towards surface deposition is avoided. Metallised microporous material obtained from this aspect of the method of the invention is stronger mechanically than the parent material itself and therefore it can be used in low temperature catalysis.

The microporous material may be a carbon based microporous polymer, a carbon or textile fibre felt, carbon fibre matt, fabric material or a microporous polyHIPE polymer. Preferably the microporous material is a microporous polyHIPE polymer. Preferably the microporous polyHIPE polymer is one which is specifically or generically described in WO-A-00/34454, in PCT patent application filed on an even date herewith claiming priority from GB0215833.5 or herein.

To establish microporous material at the steady elevated temperature, the microporous material may be initially heated. For the purposes of initial heating, the microporous material may be placed in a cell which is heated. Heating (*eg* of the cell) may be carried out electrically, using a water bath.or ultrasonic or microwave irradiation.

In a preferred embodiment, the microporous material is established at the steady elevated temperature by injecting (preferably periodically injecting) water at above room temperature (*eg* hot water for example at 95°C) or steam (*eg* at 100°C or more).

Steam is advantageous in that it condenses within the pores of the template material and releases latent heat to maintain steady the elevated temperature of the microporous material. However, hot water is advantageous to remove salts created during deposition of the metal.

In a preferred embodiment, the surface of the microporous material may be activated (*eg* using a metal such as palladium). This leads to surface deposition of the metal from the metal solution.

Preferably the metal solution is passed through the microporous material by force. This may be exerted on the metal solution mechanically (*eg* by pumping using for example a

piston) or using a compressed gas (*eg* air). If necessary where the microporous material is not mechanically strong (*eg* when fibre or felt is used), it may be supported by a plastic mesh.

Where force is exerted by pumping, the rate at which the metal solution is pumped may affect the grain size of the metallic deposition. Typically the flow rate is in the range 1 to 4 ml/minute (*eg* about 2.5ml/min).

Where the microporous material is a microporous polyHIPE polymer, it is preferred to create a metallised skin on the metallised microporous polyHIPE polymer surrounding a metallised core, wherein the metallised skin is denser than the metallised core. This may be achieved by reducing the flow rate from that which is used for uniform metallisation.

After deposition of a desired amount of metal, the metallised microporous material is useful in its own right. However in a preferred embodiment of this aspect the invention, the microporous material of the metallised microporous material constitutes a template and the process further comprises:

removing the template to produce a microporous metal.

Removal of the template may be carried out thermally. The metallised microporous material may be heat treated below the melting point of the metal. For example, the metallised microporous material may be heated in stages and heating may be carried out in an inert atmosphere (*ie* an inert gas or under vacuum). The temperature, duration of heat treatment and the environment effect the properties of the microporous metal.

Preferably heating is carried out (at least in a final stage) at a sintering temperature with the added advantage that the microporous metal gains further porosity (*eg* at high temperature such as at 1200°C for nickel). Heating may leave a microporous metal with a structure which is useful for high temperature applications or when the accessible surface area of the metal is needed. The resulting (polymer free) microporous metal may require further heat treatment for strength and to further develop its fine structure.

In order to make the microporous metal stronger, it is possible to use sintering agents or aids (which for microporous polyHIPE polymers may be added at the emulsification/polymerisation step as hereinbefore described). For example, where the microporous material to be metallised is a microporous polyHIPE polymer, a sintering aid may be dispersed/dissolved in the aqueous phase and subsequently deposited on the walls of the microporous polyHIPE polymer. For example, where the microporous material to be metallised is a microporous polyHIPE polymer, a sintering aid may be incorporated in the oil phase (preferably after coating it with a silane coupling agent to render it hydrophobic). Sintering agents will act as a glue to ensure the integrity of the metallised microporous polymer.

The microporous material may be a microporous polyHIPE polymer obtainable by filling a scaffold of highly macroporous metallic support structures (*eg* with pore size of the order 1 to 2 mm) in a 3D network (for example of steel wires) with polyHIPE polymer. This improves the strength of the subsequently metallised microporous polyHIPE polymer in accordance with this aspect of the invention. Alternatively when the microporous material is a microporous polyHIPE polymer, metallic short fibres or continuous fibres may be used to add strength (as described hereinbefore).

Another technique for adding strength to metallised microporous materials is to use a lower melting point metal powder (as described hereinbefore) which alloys with the metal of the metal solution (eg nickel) so that the metal particles are glued together during the heat treatment period. This technique is similar to the use of sintering agents used in sintered metal production.

Preferably the metal solution is a nickel, copper or chromium solution or a solution of shape memory metal. Preferred is a nickel solution.

Metal deposition may be successively repeated (*eg* in accordance with the process of this aspect of the invention or by other deposition techniques such as electrodeposition) with the same or a different metal (*eg* a nickel solution in accordance with the invention followed by chromium or copper coating). Between each metal deposition, the deposition

of an organic coating (*eg* a silane coupling agent) may be desirable (*eg* by dip coating).

Both nickel and carbon are used for the storage of hydrogen. The use of carbon with nickel for this objective can be achieved by using microporous systems which also have nano-porous features to increase the hydrogen holding capacity.

Preferably the microporous material in this aspect of the invention is a microporous polyHIPE polymer obtainable in the following steps:

adding a polymerisable high internal phase emulsion to a fibre felt; and

polymerising the polymerisable high internal phase emulsion whilst agitating (*eg* shaking) or mixing.

The metallised carbon microporous composite is an intermediate from which the fibres can be removed to yield large capillaries for accessibility as well as extended surface area whilst the microporous regions of the microporous polyHIPE polymer provide large active surface area. If the fibers are completely removed, the microporous metal is composed of micro-capillaries (pipes) whose capillary walls provide strength and integrity. The phase volume of the microporous metals can be as high as 96%.

Alternatively preferably the microporous material in this aspect of the invention is a carbon felt. Carbon/graphite fibres as a template provide several advantages. For example the porosity of the material may be as high as 96% (although this may be reduced by compressing the felt). Since the material is electrically conductive, it may be coated with a suitable metal through electrodeposition (with for example copper) and subsequently re-coated with a suitable organic material (*eg* through dip coating) in order to prevent the re-dissolution of copper during nickel deposition. Alternatively, after coating of carbon fibres with a suitable metal, it can be re-coated with a silane coupling agent. When a specific type of metal (such as chromium) is needed at a given concentration (for catalytic function) this specific metal deposition can be achieved through elecrodeposition, followed by coating with an organic material. These organic materials are available as colloidal emulsions (such as epoxy resins) where the dispersed phase size is less then 1 um. These emulsions/dispersions can also be used to deliver other desirable active ingredients such as

nano-sized metals to enhance catalytic activity.

Further alternatively preferably the microporous material in this aspect of the invention is a woven textile fabric. The process of this aspect of the invention leads to the formation of a template yielding a large number of capillaries when the fibres of the fabric are removed. These capillaries or bundle of capillaries are porous and due to the existence of relatively large capillaries, small pores are also accessible for catalytic reactions.

Yet further alternatively preferably the microporous material in this aspect of the invention is a composite polyHIPE polymer in which the inclusion is a metal fibre such as copper fibre. Such a composite polyHIPE polymer may be prepared in accordance with the first aspect of the invention.

The microporous metallised materials prepared in accordance with this aspect of the method of the invention may be produced in complex shapes and large sizes. They may be used as microreactors or heat exchangers. When shape memory metals are used, microporous shape memory structures may be prepared which can respond to changes in temperature faster. These materials can also be obtained in particulate form with the inner core containing activated carbon if desired.

From a further patentable viewpoint, the present invention relates to an apparatus which permits the method of the previous aspect of the invention to be carried out to achieve a uniform deposition of metal.

Viewed from a further aspect the present invention provides an apparatus for metallising a microporous polymer using a metal solution comprising:
a chamber for supporting the microporous polymer in a reservoir of the metal solution;
one or more first inlets for admitting metal solution to the chamber and one or more outlets for releasing metal solution from the chamber;
one or more second inlets for admitting water to the chamber at a temperature above ambient temperature; and
a forcing device for substantially uniformly forcing metal solution through the microporous

polymer.

The chamber may be defined by one or more parts of a mould which may be clamped to be waterproof. The mould may be heated (*eg* by an externally mounted heater).

Preferably the forcing device comprises: first and second pistons reciprocally mounted within the chamber so as to captivate metal solution against the microporous polymer, wherein the first and second pistons are positioned so as in use to exert a force against opposing faces of the microporous polymer. By reciprocally mounting the pistons in this manner, the apparatus is operable in a symmetrical manner to achieve uniform metal deposition.

For example, the first piston may be movable towards one face of the microporous polymer whilst the second piston may be reciprocally movable away from a second opposing face of the microporous polymer (and vice versa).

The present invention will now be described in a non-limitative sense with reference to the following examples and Figures in which:

Figures 1c, d illustrates the general structure of MCP1 and MCP2;
Figure 2a, b illustrate microcellular polymer with encapsulated urease;
Figure 3a, b illustrates a schematic diagram of the experimental system for a microbioreactor;
Figure 4a-c illustrates a relatively large pore sized MCPl slice with a pore diameter of 40μm and an interconnect diameter of 20μm immediately after the seeding procedure: **(a)** surface, **(b)** cross-section, **(c)** bottom;
Figure 5 illustrates a cross-section of the MCPl slice at the end of the continuous cultivation at 130 μm vicinity of the surface;
Figure 6 illustrates the MCP2 slice at the end of the 30-day continuous cultivation **(a)** surface (at low magnification), **(b)** surface (at high magnification), **(c)** cross-section at a distance of 2000 μm from the surface, **(d)** cross-section at a distance of 2500 μm from the surface, **(e)** cross-section at a distance of 3000 μm from the surface, **(f)** cross-section at a

distance of 4000 μm from the surface, **(g)** cross-section at a distance of 3500 μm from the surface;

Figure 7 illustrates the relationship between the distance from the top surface of MCP2 and the number of the cells. The intercept of the cracked line on the abscissa shows the optimized length of the micro-reactor;

Figure 8 illustrates the relationship between utilized phenol at the initial phenol concentration of 380 mg/l and time at the transient conditions at various flow rates ranging from 0.5-3 ml/min;

Figure 9 illustrates particles of microporous polymer with a gradient of pore size; and

Figure 10 illustrates schematically a system for intensification of *in situ* bioremediation;

Figures 11a-j illustrates various metallic microporous structures;

Figure 12 illustrates an embodiment of the apparatus of the invention for metal deposition.

Example 1: *Enzyme encapsulation and the intensification of enzymatic reactions*

An aqueous phase was prepared containing water, enzyme and optionally an aqueous phase initiator, an additional electrolyte, an enzyme stabiliser and co-enzyme. A continuous oil phase was prepared containing a monomer (such as styrene) a cross-linking agent (such as divinylbenzene), a surfactant and an oil phase initiator (if the use of an aqueous phase initiator is not desirable). Optionally, the oil phase contained non-polymerisable oils which can be leached out to increase the permeability of the walls as described in WO-A-00/34454.

Polymerisation whilst shaking was carried out in plastic tubes and subsequently the solid polymer was washed out with water and isopropanol in a flow through system. During washing, excess electrolyte, initiator, monomer or non-reactive oil were removed. There was no indication of enzyme or degraded enzyme in the wash liquors. Polymerisation was carried out at a temperature below the deactivation temperature of the enzyme. Examples of enzymes encapsulated successfully in this manner were invertase and urease at concentrations above 1g/l. The appearance of the resultant microcellular polyHIPE polymers with encapsulated enzymes is shown in Figures 2a and b.

After washing, the polyHIPEpolymer containing encapsulated enzyme was dried and stored without any loss of enzymatic activity. Such encapsulated enzymes may be used in bio-reactions in flow through systems.

Example 2: *Skin/Core Structures(Bacterial Seeding)*

In order to obtain skin/core polymers, styrene polyHIPE polymers prepared as in WO-A-00/34454 can be moulded into sheets (3 cm thick) and concentrated sulphuric acid then applied from both sides. No excess free acid is needed. The temperature is then raised to 60C or more (up to 170C maximum) and the polymer is allowed to sulphonate and subsequently neutralized and washed in the usual way. The sheets are the cut up into two 1.5 cm thick and any desired shape.

This example revealled a hydrophobic core which can be seeded with bacteria for use in agriculture, horticulture, hydroponics or biotechnology. If the hydrophobic core does not need to be exposed, suitably cut pieces of the polyHIPE polymer (for example 1 cm sized cubes) can be soaked in concentrated sulphuric acid and the temperature raised above 60C (up to 170C in order to increase the rate of sulphonation) to complete sulphonation followed by neutralization and washing.

Example 3: *Microcellular Polymers for Bacterial Support and Intensification of Biological Processes*

Nomenclature used in this Example is:

C	phenol concentration (mg l^{-1})
C_0	initial phenol concentration (mg l^{-1})
CH	outlet phenol concentration (mg l^{-1})
C_H^*	steady state outlet phenol concentration (mg l^{-1})
ΔC	phenol utilization (mg l^{-1})
ΔC^*	phenol utilization under steady state conditions (mg l^{-1})
D_R	dilution rate based on the total reactor volume (min^{-1})

D_{Ro} optimized dilution rate basing on the active reactor volume (min^{-1})

F flux rate ($1m^{-2}h^{-1}$)

H height of the column reactor (cm)

MCP macrocellular polymer

OD optical density

Q volumetric flow rate (ml min^{-1})

P_r steady state reactor productivity (g $1^{-1}h^{-1}$)

R volumetric utilization rate of phenol (g $1^{-1}h^{-1}$)

U Utilization rate (g^{-2} min^{-1})

V_i true liquid volume of the reactor (cm^3)

V_S apparent volume of the support matrix (cm^3)

V_A active reactor volume (cm^3)

V_t total volume of the reactor (cm^3)

$Y_{X/S}$ biomass-to-substrate-yield coefficient (g g^{-1})

Phenol was chosen as a model substrate to conduct continuous micro-bioreactor experiments. There have been various degradation studies using phenol and other aromatic compounds by free or immobilized cells of microorganisms (Klein and Schara, Applied Biochem. Biotechnol., 6, 1981: 91-117; Bettmann and Rehm, Applied Biochem. Biotechnol., 20, 1984:285-290; Schroder *et al.*, Biotechnol. bioeng., 54, 1997:567-576; and Hecht *et al.*, Biotechnol. Bioeng., 70, 2000: 391-399). Phenol has a highly toxic effect on all bacteria and there are various data reported on the inhibitive effect of phenol ranging between 50-350 mg/l on growth of *Pseudomonas* sp. (Hill and Robinson, Biotechnol. Bioeng., 17, 1975: 1599-1615). Furthermore, the degradation of phenol is aerobic and therefore it presents further challenges to the intensification of bioconversions. The degradation of phenol using immobilised enzymes and immobilized cells are reported elsewhere using the microporous cell support system originally developed for animal cell support in tissue engineering applications (WO-A-00/34454).

Experimental

Materials

Phenol, Styrene, DVB (Divinylbenzene), sorbitan monooleate (Span 80) and isopropanol were purchased from Merck. All other chemicals used were of the highest commercially available purity.

Methods

Preparation of Micro-cellular Polymers (MCPs)

The method disclosed in WO-A-00/34454 was used to obtain micro-cellular polymers (MCP). Figures 1c, d illustrate the internal structure of these materials.

Microorganism and Growth Medium

A phenol degrading bacteria, *Pseudomonas syringae* known as AEK1 was used in a packed-bed reactor (Erhan *et al.*, 2002a, b; Akay et al., 2002 (in press). *P. sringae* from stock cultures were seeded onto Petri dishes containing nutrient agar solid media and incubated for 48 h at room temperature. Stock culture was maintained by periodic subculture on nutrient agar slants which were stored at 4°C. The bacteria were grown in a mineral medium (see Table 1) prepared with deionised water with phenol as the sole source of carbon and energy(Cohen-Bazire *et al.*, J. Cell. Comp. Physiol., 49, 1957:25-31).

Bacterial cultures were inoculated into flasks containing 250 ml media. Microorganism acclimatization was conducted by feeding increasing amounts of phenol (starting with 100 mg/l and doubling phenol every 24 h) in a shaking incubator (200 rpm) at 28°C for three days. Absorbance of the suspended culture was measured at 340nm using a UV spectrophotometer to determine the concentration of bacteria. The same composition of synthetic medium was used as a feed solution including various phenol concentrations for continuous micro-reactor experiments. Phenol was measured according to the direct photometric method using 4-aminoantyprene (Clesceri *et al*, Standard Methods for Water and Wastewater Examination, pp 465, 20th ed., United Book Press Inc, Baltimore 1998).

Micro-bioreactor

MCP polymer slices with various pore sizes and structures were cut to a thickness of 0.5cm and a diameter of 2.3cm. The MCP slice was placed into a TEFLON^R micro bioreactor shown in Figure 3a, b. The working diameter was 2.1cm. Cells were seeded by recycling the suspended cell culture at an optical density of 1.5 ABS through the micro-bioreactor for 24 hours in a reactor configuration illustrated in Figure 3b. Two reactors in the same configuration were set to seed two MCP slices (coded as MCP1) with the same micro-architecture at the same time under the same conditions. The results of their characterisation are seen in Tables 2-4.

At the end of the seeding, one of the MCP1 slices was taken out of the reactor and cells were fixed with gluteraldehyde solution for SEM analysis to show the performance of the new seeding technique. The second MCP1 slice was kept in the micro-bioreactor to carry out continuous cultivation. A fresh liquid media including 200 mg/l phenol (Table 1) was then continuously fed through the micro bioreactor for 24 hours. Concentration of phenol was measured as a function of time. Experiments were conducted under sterile conditions. A cellulose nitrate membrane with pore diameter of 0.2μm was placed on the topside of the MCP support to avoid contamination by the MCP support of the upstream tubes connected to the inlet of the micro-bioreactor.

The procedure was repeated using another type of MCP having a different micro architecture. This support was coded as MCP2. The seeding procedure described above was replicated for MCP2 support placed in a micro-bioreactor. After seeding, the micro-bioreactor was fed with a fresh phenol solution at an initial concentration of 200 mg/l for 8 days until the phenol utilization reached a detectable level in the outlet flow of the micro-bioreactor. The same experimental procedure was then followed for each experiment at various initial phenol concentrations and flow rates. During these experiments, aeration was conducted directly at the inlet of micro-bioreactor with a syringe needle and a manually controlled valve. Dissolved oxygen values were not measured at the inlet or outlet of the reactor.

Scanning Electron Microscope (SEM)

MCP samples containing bacteria were fixed for 1 hour in a solution of glutaraldehyde (2.5 % in Sorenson-buffer pH 7.0) and then dehydrated by treating with 30, 50, 70, 80, 90 and 96% ethanol (each for 1 h). At the end of the dehydration process, the samples were treated three times for 1 h with absolute ethanol. After a critical-point drying procedure, the MCP samples were coated with gold for SEM analysis.

Theoretical Background

Phenol utilization, ΔC, is defined as:

$$\Delta C = C_O - C_H \qquad (1)$$

Under steady state conditions when $t \Rightarrow \infty$

$$\Delta C \Rightarrow \Delta C^* = C_o - C^*_H \qquad (2)$$

where C^*_H is the substrate concentration at the reactor outlet at steady state.

The steady state reactor productivity, P_r is defined as:

$$P_r = D_{Ro} \, \Delta C_H^* Y_{X/S} \qquad (3)$$

where $Y_{X/S}$ is a constant ratio of the substrate conversion to product. We assume the $Y_{X/S}$=0.7 for the signifying the *Pseudomonas sp.* grown in phenol. Various experimental values of $Y_{X/S}$ are reported in the literature ranging from 0.52 to 0.94 (Reardon *et al.*, J. Biotechnol. Bioeng., 69, 2000: 385-399). There, $D_{ro} = Q/V_A$ in which V_A is the active reactor volume given by;

$$V_t = V_i + V_S \qquad (4)$$

where V_s is the apparent volume of the support matrix and V_i is the true volume of the

liquid present in the reactor. The true dilution rate is based on the true liquid volume of the bioreactor and is defined as;

D_i=Q/V_i (5)

The steady state characteristics of the bioreactor performance are described by the observed utilization rate of phenol *(U)* basing on flux rate *(F)* and observed volumetric utilization rate of phenol *(R)* defined respectively by;

$U=F\Delta C^*$ (6)

$R=D_{Ro}\,\Delta C^*$ (7)

where F is the flux rate defined as $F=Q/A$ in which A is the cross sectional area.

Results and Discussion

Characterization of the Micro-Cellular Polymers used as Cell Support

As seen from Figure 1c, MCP1 has very large pores and interconnects and therefore passage of bacteria across the cell support material is unhindered. Furthermore, the hydrodynamic conditions within the pores can be considered to be dominantly shear deformation. Due to the large interconnect/pore size ratio (~0.5), the surface area per unit volume of support is reduced for cell adhesion. On the other hand, MCP2 (Figure 1d) has small pores and interconnect size and therefore the available surface area per unit volume is significantly large for bacterial adhesion. However, small interconnect size results in a flow regime, which has a very important extensional deformation component. Assuming that MCP2 (with small interconnect size) can be modelled as two flow domains connected through a capillary of size d (*ie* interconnect size), the magnitude of maximum shear and extension rates at the capillary entry and exit are given by (Akay, 1983; 1998)

$\Gamma_m = 32q/\pi d^3$, $\Sigma_m = 1/2\,\Gamma \sin \Phi$

where q is the flow rate and Φ is the capillary entry or exit angle which can be assumed to be 180^{o} (*ie* flat entry /exit).

The examination of the MCP structure indicates that on average there are 14 interconnects per pore and therefore we can assume that there are approximately (14/6) interconnects perpendicular to the direction of flow at the entrance or exit. Denoting the micro-bioreactor MCP diameter by D_s (D_s= 2.1cm here) and the average pore diameter by D_p, the flow rate per capillary is given by;

$$q = 14/6\ Q\ \{d_p/D_s\}^2$$

where Q is the flow rate through the micro-bioreactor. At a typical flow rate of Q = 1 cm^3 /min, the estimates of the maximum shear and extension rates for MCP2 (D_p=20 μm, d_p=5 μm) are $\Gamma \approx 20\ sec^{-1}$ and $\Sigma_m \approx 10\ sec^{-1}$. At this rate of deformation we can expect no substantial cell fracture due to flow although the elongated microorganisms are more susceptible to break up in extensional flows at the capillary exit.

Seeding of Microorganisms

The SEMs of a relatively large pore sized MCPl slice with a pore diameter of 25±2μm and an interconnect diameter of 5±2 are seen in Figure 4a, b, c immediately after the seeding procedure in the micro-bioreactor. It is observed that a small number of cells are immobilized on the top surface of MCPl in the micro-bioreactor during 24-hour continuous seeding (Figure 4a). It can also be seen that there is a small amount of cell debris on the top surface of MCPl because of the shear stress and fluid impact. Cell immobilization is present in each pore attached to the pore walls 2500 μm from the surface of MCPl (Figure 4b). Figure 4c shows that the seeded cell concentration at the bottom surface of MCP1 is also low. The SEM micrographs clearly illustrate that the seeding procedure was achieved successfully for bacterial cells by using MCP1 under pressure since the cells were able to attach to MCPl.

Cell Proliferation During Phenol Degradation Micro-Bireactor

After seeding of the cells, the micro-bioreactor was fed with a phenol solution at an initial concentration of 200 mg/l for 24 hours. At the end of cultivation, the phenol utilization was not found in a detectable range at the outlet of micro-bioreactor. At the end of this continuous cultivation, a high level of proliferation of the bacteria was observed within the polymer as seen in Figure 5 which illustrates the cross section of MCPl 130μm below the top surface. As seen in Figure 5, the cells are attached to each other indicating that the cell growth is in the exponential phase. It is clear that the cell proliferation is in monolayer and there is no sign of extra-cellular matrix production.

Figure 6a, b show the top surface of MCP2 after 30-day continuous cultivation. It can be seen that the top surface of MCP2 is fully covered by the cells but most of the interconnects are still open. After the seeding procedure, a 0.2μm pore sized membrane was placed on the top of MCP2 and continuous cultivations were carried out in the presence of this membrane. When MCP2 was taken out of the micro-bioreactor for SEM analysis, the colour of its top surface was observed to be pale brown indicating the accumulation of the cells between the top surface of MCP2 and the membrane.

The cross sections of MCP2 at various depths are illustrated in Figures 6c-h. It can be seen that extremely good cell proliferation is present when the distance from the top surface is less than ~2500 μm (Figures 6c, d). At a depth of 2500μm, the cell coverage begins to decrease gradually through the cross section of MCP2 as seen in (Figure 6e, f). Once again the cells appear to establish a monolayer on the surface of the pore walls without any sign of extra-cellular matrix formation. From the SEM micrographs, it was observed that the cells first proliferate in the regions away from the interconnecting holes which lay perpendicular to the direction of flow. It is also observed that some of the interconnect holes are surrounded preferentially first by the cells compared with the interconnects which provides the flow channels. Therefore it can be concluded that the cell proliferation and cell adhesion to the pore walls are affected by the flow conditions. The distribution of bacteria concentration as a function of distance from the top surface is shown in Figure 7. This figure was evaluated by counting the number of bacteria on a given surface area at various depths. It can be seen that the microorganism number density is maximum on the

top surface but it remains constants over a depth of ≈2500 μm and subsequently decays rapidly.

Micro-Bioreactor Performance

Phenol was used as a model substrate to follow cell proliferation inside the polymer and the intensified degradation. Continuous experiments were conducted at various flow rates ($0.5 \leq Q \leq 4$ ml/min) and initial phenol concentration, C_o in the range $50 \leq C_o \leq 650$ mg/l. Optical density of the cells in the outlet flow of the micro-bioreactor was measured as an indicator of cell wash. The system was able to reach the steady state in a very short time (within 5 minutes for each experiment). Results are given in Table 4 which illustrate the performance of the micro-bioreactor. It can be seen that 380 mg/l phenol in the feed solution is degraded completely in a single pass through the reactor when the flow rate is Q=1 or 2 ml/min (Figure 8). A high cell wash was observed during these continuous cultivations. It can also be seen that increased flow rate at the initial phenol concentration of 380 mg/l to the level of 4 ml/min results in decrease of phenol degradation. At the highest phenol inlet concentration of 650 mg/l used in the experiments, the outlet phenol concentration was 350 mg/l at the flow rate of 2 ml/min after one pass. The washing of cells was evidence of cell proliferation in the microbioreactor. The level of optical density in the outlet flow shows that the washing of cells increases with the increasing amount of degraded substrate (Table 5). When the initial concentration of phenol is about 100 mg/l or less (50 mg/l), the optical density is at a level of 0.1 or less.

The current phenol degradation rates are found to be extremely high compared with those reported in the literature. This is primarily the result of very high cell concentrations and the reduced diffusion mass transfer resistance. In the first instance, this might be achieved through the reduction of the diffusion path for the substrate and metabolites by decreasing the mass transfer resistance and through the building of a suitable environment for the microorganisms. Also, the use of MCPs as a cell support in this novel seeding technique followed by pressure driven reactor operation resulted in an intensified micro-bioreactor system partly due to the very high surface area of the support and partly due to the miniaturization in the reactor design. Consequently, the degradation of phenol per unit

bioreactor volume was found to be very high caused by the use of this newly introduced micro-bioreactor system.

In the present technique, where bacteria are immobilized within the pores of the MCPs subsequently used as a micro-bioreactor in monolithic form, control of bioreaction can be carried out at a microscopic level. The reactor volume is drastically reduced thereby providing all the advantages of classical Process Intensification technology. This involves the building of a micro-bioreactor system with a well-defined micro-architecture and surface/bulk chemistry as bacterial support in the form of cartridges. It was found that the bacterial proliferation within the pores of the monolithic support resulted in monolayer coverage with apparently no extra-cellular matrix.

Example 4: *Preparation of Microporous Polymers with a Gradient of Pore Size*

A network of capillaries was created within the bulk of a polyHIPE polymer from a composite polyHIPE polymer. In a specially constructed mold, a 3D network of fibres are constructed (either in 1, 2 or 3 directions) and the remaining space is filled with a high internal phase emulsion which can include functional inclusions in the dispersed phase. Polymerisation is conducted whilst shaking the mold. After polymerisation, the fibres are pulled out or dissolved in acid (for metallic fibres). The remaining bulk polymer can then be granulated to desired size to obtain powdered microporous material with a network of capillaries. Alternatively, they can be cut into suitable size pieces as packing materials. Such packing materials may also be useful in other separation processes such as distillation and liquid liquid extraction. If the fibres used in obtaining the capillary network are hydrophilic, the capillary pore wall interface will have an open, interconnected pore structure.

Alternatively, short fibres may be dispersed in the high internal phase emulsion and polymerisation is carried out by shaking. After completion of polymerisation, the bulk polymer is granulated and fibres are dissolved in a solvent or in an acid to leave a microporous polymer with a gradient of pore size. This technique is useful when the granular powder is sulphonated or nitrated in order to obtain porous ion exchange resin in

which concentrated acid is used for the sulphonation or nitration (as disclosed in PCT patent application filed on an even date herewith claiming priority from GB0215833.5).

Example 5: Double HIPE Polymerisation

A cost effective method for obtaining powdered microcellular polymers is to use a double HIPE polymerisation process. An emulsion prepared as in WO-A-00/34454 is re-dispersed into water using a more hydrophilic surfactant than that used in the formation of the high internal phase emulsion with a hydrophile-lipophile balance (HLB) in the range 8 to 15. After dispersion, polymerisation is conducted whilst mixing. The resulting dispersion contains porous microcellular polymeric particles. These particles appear to have in them very large pores extending through the particle like a capillary. Such particles are shown in Figure 9.

Example 6: Incorporation of Nano and Sub-micron Particles

Carbon black was placed into an aqueous phase which was subsequently mixed with an oil phase containing a monomer and a surfactant. The temperature was increased during shaking (in an incubator shaker) to the polymerisation temperature and the shaking was conducted at 120rpm. The resulting composite microporous polymer was mechanically much stronger than the microporous polymer in the absence of carbon black. If this material is sulphonated, the resulting ion exchange material is dimensionally stable (does not swell) in water.

Example 7: Application of Microporous Polymers to in situ Soil Re-mediation

Electroosmosis may be used to transport toxins and metal cations to a porous cathode 1 as shown in Figure 10. A porous anode 2 and the cathode 1 in Figure 10 are made of porous carbon. This porous cathode 1 is surrounded by a cation exchanger, sulphonated microporous co-polymer (prepared in accordance with a PCT patent application filed on an even date herewith claiming priority from GB0215833.5) 3a, 3b which acts as a metal ion adsorption barrier. Any remaining metal ions are deposited on the electrode 1.

The organic toxins then pass through a non-ionic barrier 4a, 4b, to a bio-reactor 5 which contains either encapsulated enzyme or bacteria supported by micro-porous polymers. Water from the bioreactor is then recirculated back to the anode 2 as illustrated in Figure 10. Both the anode 2 and the cathode 1 and its supporting compartments (cation exchanger 3a, 3b, non-ionic barrier 4a, 4b and the bioreactor 5) can be inserted into the soil and periodically removed when each component functions efficiently.

Example 8: Metallization of Microporous Structures

In so-called 'electroless' nickel deposition, suitable nickel salts are used in the presence of hypophosphite ions in an aqueous solution and an addition agent. The addition agent serves two purposes, namely (1) formation of a complex with nickel ions to prevent precipitation of basic nickel compounds when the pH of the solution is increased and (2) to serve as a buffer to keep the pH of the solution from decreasing rapidly as the reaction proceeds (thus stabilising the reaction). Carboxylic acid or a salt of a carboxylic acid which performs at least one of these functions are used as stabilizers. Under correct conditions of temperature, pH and concentration, the reaction between the nickel ion and the hypophosphite ion (the reducing agent) is catalyzed by the presence of specific metals such as nickel or palladium. Thus after the initial nickel deposition, the reaction becomes autocatalytic and proceeds on the surface rather than in a spontaneous manner in the bulk solution. These two factors makes it possible to produce relatively thick deposits of nickel-phosphorous alloy by chemical reduction.

In any 'electroless nickel' solution formulated to meet the above requirement, the main reactions which occur are:

$NiSO_4 + NaH_2PO_2 + H_2O \Rightarrow Ni + NaH_2PO_3 + H_2SO_4$ (Catalytic reaction)
$NaH_2PO_2 + H_2O \Rightarrow NaH_2PO_3 + H_2$ (catalytic reaction).

Thus as the reaction proceeds, the solution becomes more acidic and the hypophosphite is oxidised to an acid phosphite as the nickel is reduced to metallic form. Simultaneously, a

certain amount of sodium hypophosphite reacts with water and yields acid phosphite with the liberation of hydrogen.

If the reaction is carried out in alkaline conditions, there is no need for a catalyst, since:
$H_2PO_2^- + H_2O \Rightarrow H_2PO^-_3 + H_2$

In the presence of ions of heavy metal such as nickel, cobalt, copper, silver, palladium, radium, gold etc, reduction of the metal takes place:

$M^{+2} + H_2PO_2^- + H_2O \Rightarrow M + 2H^+ + H_2PO^-_3$

Concentration of nickel ions in the solution can be increased by using complexing agents such as sodium pyrophosphate. Ammonium hydroxide is used to adjust the pH of the solution to 9-12. Typically, nickel bath solution contains 5 g $NiCl_2$, 300 ml water, 20ml sodium hypophosphite and 40 ml NH_3 solution.

It has been discovered that non-catalytic surface reactions can be conducted within the pores or on the surface of a microporous polymer provided that the solution is passed through the polymer which is kept at 90°C. The diameter of the porous template is 2.8 cm. Nickel solution which is kept at room temperature is pumped at 2.5 ml/min. When the reaction starts, large amounts of hydrogen gas evolve which is carried by the solution. After the deposition of a desired amount of metal, the microporous template of the metallised microporous material is removed and the resulting microporous metal may be subjected to further heat treatment.

Example 9: *Preparation of Micro-porous Disk Bio-reactor*

Basic Experimental Procedure

Microporous polymers were prepared through a high internal phase emulsion polymerization route using the basic experimental procedure given in WO-A-00/34454.

These PHPs were obtained after the polymerisation and crosslinking of a high internal phase emulsion. In this basic procedure, the continuous phase (45 ml) which eventually undergoes polymerisation and crosslinking contained 15 wt % surfactant (Span 80), 70wt% monomer (styrene) and 15 wt% crosslinking agent (divinyl benzene). This mixture was placed at the bottom of a mixing vessel (internal diameter 12 cm) and 255 cm^3 dispersed phase (an aqueous solution containing desired amount of solute or water dispersible inclusions and 0.5% potassium persulphate) was dosed into the continuous phase while mixing. Potassium persulphate was used as the aqueous phase initiator. The phase volume of the dispersed phase was 85%.

Mixing was conducted using 3 flat paddles (diameter was 9 cm) stacked at right-angles to each other and the bottom impeller was as close to the bottom of the vessel as possible. Rotational speed of the impellers was 300 rpm. Dosing of the dispersed phase was 10 minutes and the additional mixing time was changed in order to obtain polymer with a given pore size.

After the preparation of the emulsion, it was transferred to a plastic mold of desired diameter and thickness (12 cm diameter and 2 cm thickness) and polymerised at 60 C for 6 hours to complete the polymerisation. Afterwards, the mold was opened and the polymerised material was removed. It is also possible to use perforated mold which is sealed at the time of polymerisation to prevent emulsion escape. In this case, the mold can act as a permanent holder for the porous disk. An alternative method is to use large containers during polymerisation and subsequently cut the porous polymer into desired shape and size. After polymerization, the temperature of the polymer is raised to at least 150 C or more preferential 160 C to evaporate the aqueous phase and to concentrate the acid contained in the aqueous phase. This also resulted in sulphonation (the degree of sulphonation being dependent on the sulphonation temperature and time). Sulphonation at 150 C for two hours results in the production of hydrophilic polymer. This polymer can then be washed in water and isopropanol to remove excess acid, residual monomer and the surfactant (Span 80). If the sulphonation is carried out at or below 140 C, the resulting polymer does not adsorb water after washing.

Enzyme Experimental Procedure

The basic experimental procedure was repeated but using a different internal phase. In this example, 5g enzyme (urease) was dispersed in one litre of water containing 0.5 g potassium per sulphate. 45 ml of oil phase (the same as in the basic procedure) and 255 ml of this aqueous phase were mixed to obtain an emulsion as described in the basic procedure. The emulsion was placed in a cylindrical container and placed horizontally into a shaking incubator at 60°C. The incubator was allowed to shake the emulsion at 120 rpm. The container was not full to allow the emulsion to be agitated during polymerisation while shaking. This was continued for 2 hours and the polymerisation was completed under static conditions. After polymerisation, polymer was cut up into desired size and washed in water and isopropanol to remove residual monomer, surfactant and potassium sulphate.

Microencapsulated enzymes are free within the micropores but are unable to leave the pores since the size of the interconnects are smaller then the size of the enzyme molecules. When the polymer containing urease is dried for examination under scanning electron microscope, clusters of the enzyme were observed. The structure of the pores and the presence of enzymes are shown in Figures 2a, b. This method of enzyme encapsulation results in high enzyme concentration without chemical bonding of the enzymes to the support material.

Example 10: *Inclusion of Fibres in Micro-porous Disk Reactors*

The emulsification carried out in the basic procedure set out above was essentially repeated. However, the aqueous phase contained no acid (*ie* contained water and potassium persulphate only). After the formation of 300ml emulsion (85% aqueous dispersed phase and 15% oil phase) with 20 minutes mixing time, 4g of chopped carbon fibre was added gradually while continuing to mix. Mixing was continued for a further 5 minutes. The emulsion was transferred to a polymerisation container and placed in a shaking incubator at 60°C. Shaking during polymerisation was continued for 2 hours at 120 rpm. After another 6 hours of polymerisation, the resulting polymer was cut into a desired size and washed in water and isopropanol to remove un-reacted monomer, surfactant and potassium sulphate

generated during the decomposition of potassium persulphate. The polymer was examined under a scanning electron microscope.

Example 11: *Metallisation*

Metal deposition can be carried out in an apparatus 1 which is illustrated in Figure 12 using the experimental procedure set forth below. In this apparatus, various types of microporous material were metallised. In this Example we refer to the microporous material used in metallization is referred to as a template material.

The apparatus 1 is symmetrical so that the deposition of nickel can be conducted either from the top or from the bottom. The apparatus is made from PTFE plastic in order to provide dimensional stability and resistance to high temperature. The template material A measuring 2.8cm in diameter and 3mm thickness was placed in a chamber generated by two halves M and N of a mould which are clamped together to form a waterproof system. An electrical heater L is placed around the mould. Each half M and N of the mould has two inlets F, G, H and I and a piston to force the solutions through the template material. Inlets H and I are used to take in the stock nickel solution (they are used one at a time) while inlets I and G are used to inject high temperature (90-95 C) water to maintain the temperature of the template A. Two additional outlets J and K are used to drain the nickel solution once it passes through the template material A. It is also possible to use steam in order to maintain the temperature of and periodically wash the template material and for this purpose a small steam generator may be used.

A uniform distribution of the nickel and the washing solutions when they pass through the template can be achieved by using a piston pump D and E. Pistons D and E are driven by a variable speed motor and they are synchronized to operate in tandem, while piston D is moving forward, piston E moves backwards. There are two thermocouples R and S close to the surface of the template material A.

In a typical operation, the apparatus 1 is heated up to about 70C. Hot water (at 95C) or

steam at 110 C is then passed through the template with excess water exiting the outlets J or K. When the temperature reaches 95C, nickel solution (at room temperature) is taken into the reservoir B and subsequently forced through the template material and allowed to drain from the outlet K. When the temperature of the template drops below 85C, piston E moves upwards to drain the spent nickel solution and the piston D moves upwards while letting heating/washing liquid from the inlet J. Reservoir B is then activated to heat-up the template material followed by filling of the reservoir C with the nickel solution to be pumped upwards and the cycle is repeated. This procedure is repeated until approximately 2.5g nickel is deposited. It is also possible to inject steam into the reservoir C while the nickel solution is pumped from the reservoir B. The symmetrical operation ensures a uniform nickel deposition. Typically, the flow rates of the nickel solution and the washing solution were 2.5 ml/min (although they may be varied).

The following were used as template materials in order to exemplify the invention.

(a) 90% void rigid polyHIPE polymer (oil phase composition by weight: 78%styrene monomer, 8% divinyl benzene, and 14% surfactant Span 80) with an average pore size of 60 μm was prepared using the technique described in WO-A-00/34454 in the form of disks (3 mm thick and 28 mm in diameter). They were washed in water and isopropanol and dried at 100 C (see Figure 11a).

Samples were placed into the chamber of the apparatus 1 and metal deposition was achieved with repeated washing/heating and delivering a nickel bath solution which consisted of: 5 g NiCl2,300 ml water, 20ml sodium hypophosphite and 40 ml NH_3 solution (to adjust the solution pH to 11). Nickel deposition was carried out from both ends. 350 ml of solution was passed to obtain 2.5 gram of nickel. Each cycle consisted of passing 25 ml nickel solution (10 minutes) followed by washing/heating for 5 minutes.

This material was examined under a scanning electron microscope (Figures 11b and 11c) after coating it with carbon. Figure 11b and 11c indicate that deposition was uniform and followed the contours of the pores. Figure 11c indicates that the disc had a denser layer of

nickel.

The material was then heated to 600C in air for 2 hours and subsequently examined under SEM (Figure 11d) which indicated that the integrity of the material was maintained. Closer examination of the particles indicates the deposited particles had secondary pores (with size ranging from 100 -1000 nm) which could not be seen before burn off.

Sample from the above was then annealed at 800C and also at 1000C followed by cooling at room temperature and then examined under SEM (Figure 11e – 800C and Figure 11f – 1000C). These examinations indicated that the annealing at these temperatures did not reduce the secondary pore structure significantly. However, x-ray analysis indicated that as the temperature is raised from 600 to 800 and to 1000C, oxygen content of the metal increased as nickel oxide content increased.

Increasing metal oxide results in the loss of strength and therefore the annealing should be conducted in an inert media such as nitrogen. Any nickel oxide formed during burning process can be reduced back to nickel by annealing in a hydrogen/carbon monoxide environment above 300C. This can in fact be achieved by placing these microporous nickel disks in the gas stream produced through the gasification of for example biomass. Such gas streams contain some 10 % hydrogen and 15% carbon monoxide and no oxidising agent is present When this material is used for the catalytic cracking of tars in gas streams produced by gasification of biomass or waste, conversion of nickel oxide to nickel will be achieved *in situ*.

In order to prevent small catalyst particles from escaping into the gas stream, it is advisable to have a skin layer around the microporous nickel structure. This skin is denser than the material in the core. This can be achieved by reducing the flow rate during deposition thus allowing more time for nickel to deposit on the surface. The thickness of the dense skin can range from 25 um to 100 um (Figure 11c).

(b) Carbon felt is used as the template material. This material is often used as a porous

electrode in removing metal ions from water by electrodeposition. Graphitised carbon felt was obtained from Donac. Surface area was 9400 square meter per cubic meter. The diameter of the carbon was 13 um. This material was cut into 28 mm diameter disks with 3 mm thickness and nickel deposition was carried out. After deposition was complete, it was burnt in air at 900C and subsequently examined under an electron microscope. Due to incomplete burning, the resultant structure indicated that some graphite fibers were present with a coating of nickel. Where the fibres are burnt, micro-capillaries are obtained which appear to have porous walls. These are illustrated in Figure 11g.

(3) Any type of woven textile fabric may be used as a template. In this case, layers of textile fabrics are constructed and subsequently sewn together to provide strength in the transverse direction. The fabrics used in this example were supplied by Baltex (codePA 66-210-168/CF). They were cut into the desired shape and size and placed in to the nickel deposition apparatus 1. Nickel deposition was carried out as above. After completion of deposition, the material was burnt in air at 900 C and the resulting material was examined under scanning electron microscope. The results are shown in Figure 11h and indicate the formation of a large number of capillaries formed when the fibres of the fabric are burnt. These capillaries or bundle of capillaries are porous and due to the existence of relatively large capillaries, small pores are also accessible for catalytic reactions.

(4) Styrene based emulsion containing 85% water was prepared as above to which was added 2.5wt% of chopped copper fibre. This was polymerised at 60C with shaking. This material was subsequently dried and washed and nickel was deposited as above. After nickel deposition, it was dried. Part of the nickel and copper containing polymer was burnt off at 600C for two hours in air. This sample was subsequently annealed for 1 hour at 900 C and cooled in air.

Fig. 11i shows the presence of copper fibres after heat treatment at 600 C. Annealing at 900 C appear to result in the melting of copper fibres and imparting of a secondary structure on the nickel particles as shown in Figure 11j.

Table 1

Solution A	Concentration	**Solution B**	Concentration
Na_2HPO_4	141,2g/l	$MgSO_4$	14.45g/l
KH_2PO_4	136g/l	$CaCl_2.2H_2O$	3.33g/l
Solution C		$(NH_4)_6Mo_7O_{24}.4H_2O$	9.25mg/l
$ZnSO_4.7H_2O$	60 g/300 ml	FeSO4.7H_2O	99mg/l
Metal 44		Metal 44	5Oml/l
$ZnSO_4.7H_2O$	10,95 g/l		
$MnSO_4.7H_2O$	1,54g/l	**1 liter of MSB**	
$FeSO_4.7H_2O$	5.00g/l	Solution A: 40 ml/l	
$CuSO_4.5H_2O$	392g/l	Solution B: 20 ml/l	
Co $(NO_3)_2.6H_2O$	248g/l	Solution C: 5 ml/l	
$Na_2B_4O_7.10H_2O$	177mg/l	**Phenol**	Variable

Table 2 Characterization of the clean micro-cellular polymers used as bacterial support in the micro-bioreactor studies

MCP Code	Phase Volume Fraction	Density (g/cm^3)	Evolution from SEM	
			Pore size (μm)	Interconnect size (μm)
MCP1	90/100	0.054	25±2	5±0.5
MCP2	85/100	0.050	20±2	5±0.5

Table 3 MCP1

Location	Seeded cell concentration (no of cells/ 100 μm^2)	Cell concentration after 24 hours operation (no of cells/ 100 μm^2)
Top Surface	1	1
130 µm	10	85
Bottom surface	3	5

Table 4 MCP2

Distance from the top surface (μm)	Cell number density after 30 days operation (no of cells/ per 100 μm^2)
0	225
200	160
1000	150
1500	150
2000	150
2500	140
3000	40
3500	12
4000	8
4500	7
5000	7

Table 5

Q Flow Rate (ml min^{-1})	D_{Ro} Dilution Rate (min^{-1})	C_o Initial phenol concentration (mg l^{-1})	ΔC* Phenol utilization (mg l^{-1})	OD Optical Density of bacteria in the outlet (ABS unit)	P_r Productivity (g l^{-1} h^{-1})	U Utilization rate (mg m^{-2} min)	F Flux rate (l m^{-2} min^{-1})
0.5	0.44	50	50	0.03	0.92	72	1.44
0.5	0.44	100	100	0.1	1.85	144	1.44
1	0.88	100	100	0.1	3.69	289	2.89
1	0.88	380	380	0.4	14.04	1098	2.89
2	1.75	380	380	0.5	27.93	2196	5.78
3	2.63	380	250	0.7	27.61	2167	8.67
2	1.75	650	300	0.5	22.05	1734	5.78

Table 6

	Q ($l\ h^{-1}$) Flow rate	C_o ($mg\ l^{-1}$) Initial substrate concentration	U ($g\ m^{-2}min^{-1}$) Utilization rate	R ($g\ l^{-1}\ h^{-1}$) Volumetric utilization rate	Reactor type	F ($lm^{-2}min^{-1}$) Flux rate	A Cross sectional area of the reactor (cm^2)	H Height of the reactor (cm)
Shim and Yang (1999)	Batch (Benzene)	135	-	0.01	Fibrous-bed	Batch	11	45
Shim and Yang (1999)	Batch (Toluene)	201	-	0.02	Fibrous-bed	Batch	11	45
Akay et al. (2002)	-	500	0.78	-	membrane-immobilized enzyme	2.76 (cross-flow permeate flux)	176	0.3
Erhan et al. (2002b)	0.27	720	8.43	1.33	MCP-immobilized bacteria	15	3.01	38
Erhan et al. (2002b)	0.6	200	5.54	1.28	MCP-immobilized bacteria	33	3.01	38
Erhan et al. (2002b)	1.1	200	1.03	0.24	MCP-immobilized bacteria	60	3.01	38
Erhan et al. (2002a)	-	467	0.092	-	membrane-immobilized enzyme	0.56 (dead-end permeate flux)	45.3	-
Hecht et al. (2000)	13.32	39	0.93	0.24	Three-phase fluidized bed reactor	23.80	93.26	30
This	0.12	650	1.73	31.50	Micro-bio	5.78	3.46	0.5

study					reactor			
This study	0.12	380	2.19	39.9	Micro-bio reactor	5.78	3.46	0.5
This study	0.18	380	2.16	39.45	Micro-bio reactor	8.67	3.46	0.5

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CLAIMS

1. A process for preparing a composite polyHIPE polymer comprising:
(a) adding together a first phase and a second phase, wherein the first phase and the second phase are immiscible and either the first phase or the second phase is polymerisable;
(b) adding to one or more of the first phase, the second phase or the high internal phase emulsion one or more inclusions;
(c) causing the formation of a high internal phase emulsion;
(d) causing the onset of polymerisation of the high internal phase emulsion; and
(e) agitating the high internal phase emulsion at least after the onset of polymerisation.

2. A process as claimed in claim 1 wherein the first phase is continuous and the second phase is dispersed.

3. A process as claimed in claim 1 or 2 wherein the polymerisable phase comprises a styrene monomer or a styrene co-monomer and optionally a cross-linking agent.

4. A process as claimed in any preceding claim wherein the polymerisable phase comprises a styrene/ethyl hexylacrylate co-monomer or styrene and divinyl benzene cross-linking agent.

5. A process as claimed in any preceding claim wherein the composite polyHIPE polymer is a hydrophilic composite polyHIPE polymer.

6. A process as claimed in any preceding claim wherein step (e) is carried out at least until gelation.

7. A process as claimed in any preceding claim wherein the inclusion is selected from one or more of the group consisting of small particles, molecular particles, microscopic particles, colloidal particles, submicron particles, nanoparticles, enzymes, bacteria,

bacterial spores, fibres, metal species and cells.

8. A process as claimed in claim 7 wherein the fibres are metal, carbon, polymeric or natural fibres and are added in step (b) to the emulsion after step (c) and step (e) is carried out at least until gelation.

9. A process as claimed in claim 8 further comprising: (f') removing the fibres from the composite polyHIPE polymer.

10. A process as claimed in claim 8 or 9 wherein step (b) is carried out in a mould in which a 3D network of the fibres are constructed.

11. A process as claimed in claim 8 or 9 wherein the fibres are in step (b) dispersed into the emulsion and the process further comprises: (f') removing the fibres from the composite polyHIPE polymer.

12. A process as claimed in claim 7 wherein the submicron particles or nanoparticles are sintering agents, elemental metal, carbon black, colloidal silica, carbon, glass or combinations thereof.

13. A process as claimed in claim 1 wherein the inclusion is a colloidal or submicron particle or nanoparticle and step (e) is carried out at least until gelation.

14. A process as claimed in claim 1 wherein the inclusion is an elemental metal or a sintering agent coated with a silane coupling agent which is added in step (b) to the emulsion after step (c) shortly before gelation.

15. A process as claimed in claim 1 wherein the inclusion is carbon black or silica which is added in step (b) to the second phase during or after step (c), wherein the second phase is an aqueous phase and the first phase is a continuous phase.

16. A process as claimed in any preceding wherein the composite polyHIPE polymer is a composite sulphonated polyHIPE polymer.

17. A process as claimed in claim 1 wherein a precursor to a small particle is added in step (b) to the second phase before step (c) and the process further comprises:
(f) converting the precursor to a small particle into a small particle.

18. A process as claimed in claim 17 wherein the second phase is aqueous and in step (b) the precursor dissolves therein, wherein step (f) is carried out by precipitation.

19. A process as claimed in claim 1 wherein the inclusion is an enzyme and the enzymes in the composite polyHIPE polymer are substantially free.

20. A process as claimed in claim 19 wherein the enzyme is added in step (b) either to the second phase which is a dispersed phase optionally containing a co-enzyme or enzyme stabiliser or to the first phase which is an oil.

21. A process as claimed in claims 19 or 20 further comprising: functionalising the composite polyHIPE polymer.

22. A process as claimed in claim 21 wherein the step of functionalising the composite polyHIPE polymer comprises: immobilizing the enzyme.

23. A method for enzymatically catalysing a reaction comprising:
(1) preparing a composite polyHIPE polymer in a process as defined in any preceding claim wherein the inclusion is an enzyme or obtaining a fibre entrapped enzyme; and
(2) effecting mass transfer of substrates or products by forced convection.

24. A method as claimed in claim 23 wherein step (1) comprises preparing a composite polyHIPE polymer in a process as defined in any of claims 1 to 24 wherein the inclusion is an enzyme and step (2) comprises: (2) effecting mass transfer either by compressing/decompressing the composite polyHIPE polymer or oscillating the pressure or flow rate in a flow through assembly.

25. A method as claimed in claim 23 wherein step (1) comprises: preparing a composite polyHIPE polymer in a process as defined in any of claims 1 to 22 in the shape of a disc with enzymes immobilised on the surface and encapsulated or immobilised in the pores and step (2) is carried out by rotating the disc whilst the substrate solution is fed from the centre of the disc.

26. A method as claimed in claim 23 wherein step (1) comprises: preparing a composite polyHIPE polymer in a process as defined in any of claims 1 to 24 in the shape of a disc with enzymes immobilised on the surface and encapsulated or immobilised in the pores and step (2) is carried out by rotating the disc whilst the substrate solution is fed from the top surface of the disc.

27. A method for cultivating bacteria comprising:
forced seeding bacteria into a high internal phase emulsion polymerisable into a polyHIPE polymer or into the micropores of a polyHIPE polymer; and
allowing the bacteria to colonise the polyHIPE polymer.

28. A method as claimed in claim 27 further comprising: causing the onset of a bioreaction.

29. A method as claimed in claim 28 wherein the bioreaction is a flow-through bioreaction.

30. A method as claimed in any of claims 27 to 29 wherein the polyHIPE polymer has a

structure with a hydrophobic core and a hydrophilic skin.

31. A method for cultivating cells comprising:

forced seeding cells into a high internal phase emulsion polymerisable into a polyHIPE polymer or into the micropores of a polyHIPE polymer; and

allowing the cells to colonise the polymer.

32. Use of a composite polyHIPE polymer in bioremediation as a non-ionic barrier to organic toxins, wherein the inclusion is bacteria or an enzyme.

33. Use as claimed in claim 32 in bioremediation carried out on water from soil.

34. Use as claimed in claim 32 or 33 wherein the composite polyHIPE polymer is in monolithic or particulate form with large capillary channels.

35. A process for preparing a polyHIPE polymer comprising:

adding together a continuous phase and an aqueous phase, wherein the continuous phase and the aqueous phase are immiscible and the continuous phase contains a first surfactant and is polymerisable;

causing the formation of a high internal phase emulsion;

redispersing the high internal phase emulsion into a second surfactant, wherein the hydrophilicity of the second surfactant is greater than the hydrophilicity of the first surfactant;

causing the onset of polymerisation of the high internal phase emulsion into a polyHIPE polymer; and

agitating the high internal phase emulsion at least after the onset of polymerisation .

36. A process as claimed in claim 35 wherein the hydrophile/lipophile (HLB) balance of the second surfactant is in the range 8 to 15, particularly preferably 9 to 14, more particularly preferably 10 to 13, especially preferably about 12.

37. A process for metallising a microporous material comprising:

establishing the microporous material at a substantially steady elevated temperature; and

passing metal solution though the microporous material.

38. A process as claimed in claim 37 wherein the microporous material is a carbon based microporous polymer, a carbon or textile fibre felt, carbon fibre matt, fabric material or a microporous polyHIPE polymer.

39. A process as claimed in claim 37 or 38 wherein the microporous material is established at the steady elevated temperature by injecting water at above room temperature or steam.

40. A process as claimed in any of claims 37 to 39 wherein the metal solution is passed through the microporous material by force.

41. A process as claimed in any of claims 37 to 40 wherein the microporous material of the metallised microporous material constitutes a template and the process further comprises:

removing the template to produce a microporous metal.

42. A process as claimed in claim 41 wherein the template is removed thermally and heating is carried out at least in a final stage at a sintering temperature.

43. A process as claimed in any of claims 37 to 42 wherein the metal solution is a nickel solution.

44. A process as claimed in claim 37 wherein the microporous material is a microporous polyHIPE polymer obtainable in the following steps:

adding a polymerisable high internal phase emulsion to a fibre felt; and

polymerising the polymerisable high internal phase emulsion whilst agitating or

mixing.

45. An apparatus for metallising a microporous polymer using a metal solution comprising:

a chamber for supporting the microporous polymer in a reservoir of the metal solution;

one or more first inlets for admitting metal solution to the chamber and one or more outlets for releasing metal solution from the chamber;

one or more second inlets for admitting water to the chamber at a temperature above ambient temperature; and

a forcing device for substantially uniformly forcing metal solution through the microporous polymer.

46. An apparatus as claimed in claim 45 wherein the forcing device comprises:

first and second pistons reciprocally mounted within the chamber so as to captivate metal solution against the microporous polymer, wherein the first and second pistons are positioned so as in use to exert a force against opposing faces of the microporous polymer.

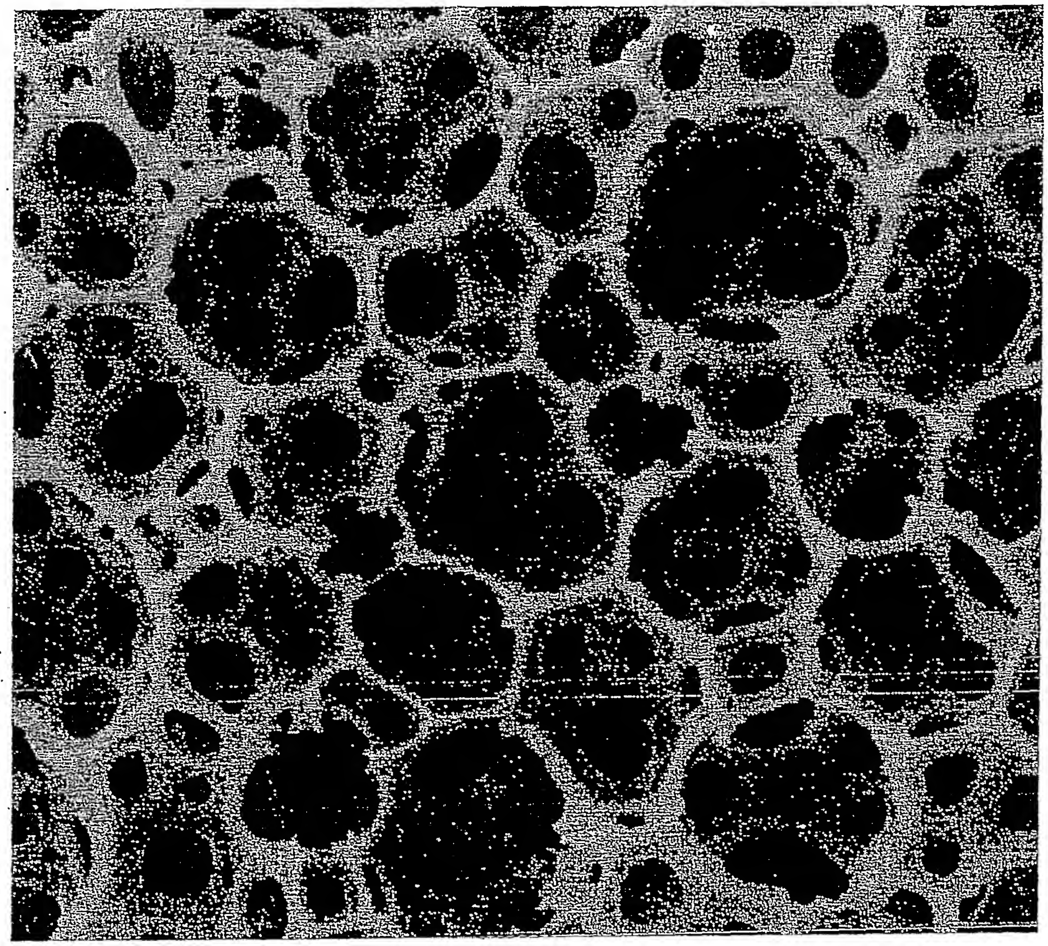


Fig. 1 (a)

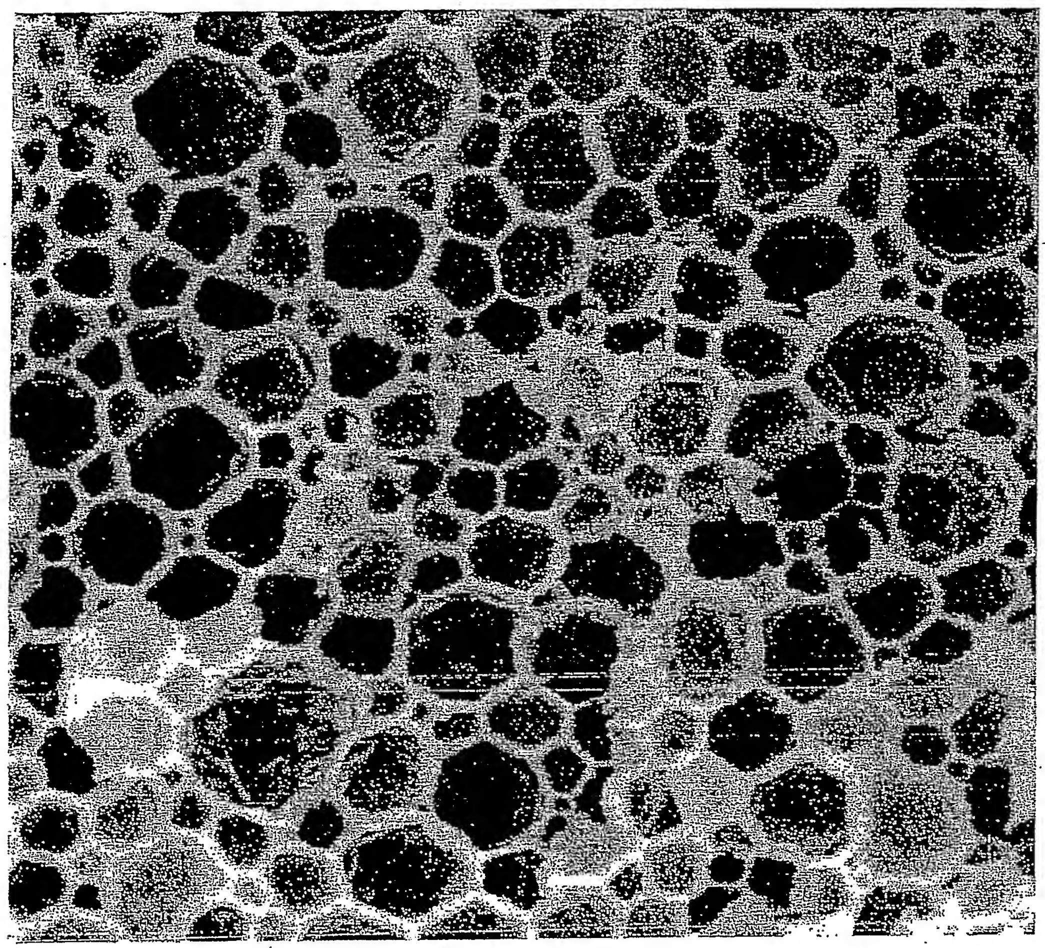


Fig. 1 (b)

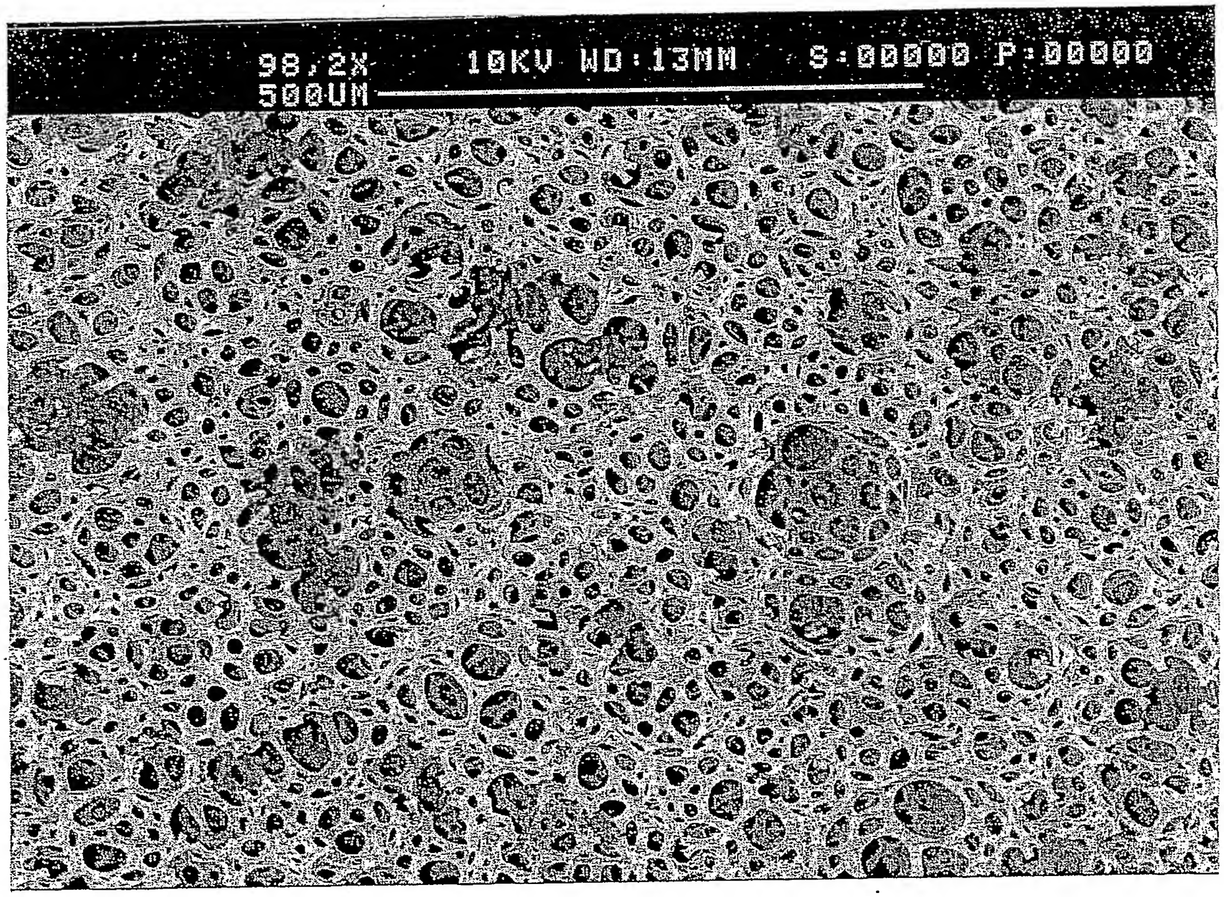


Fig. 1 (c)

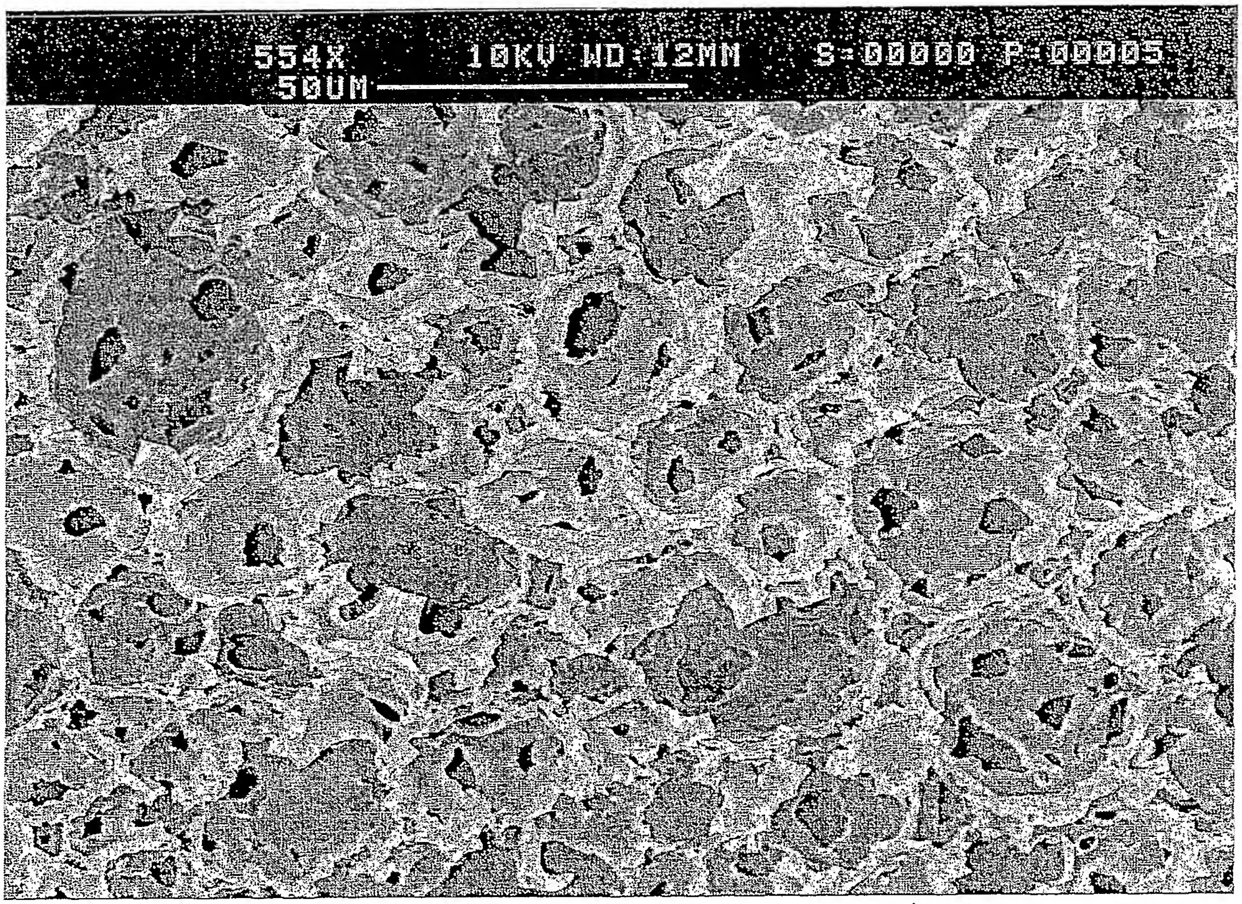
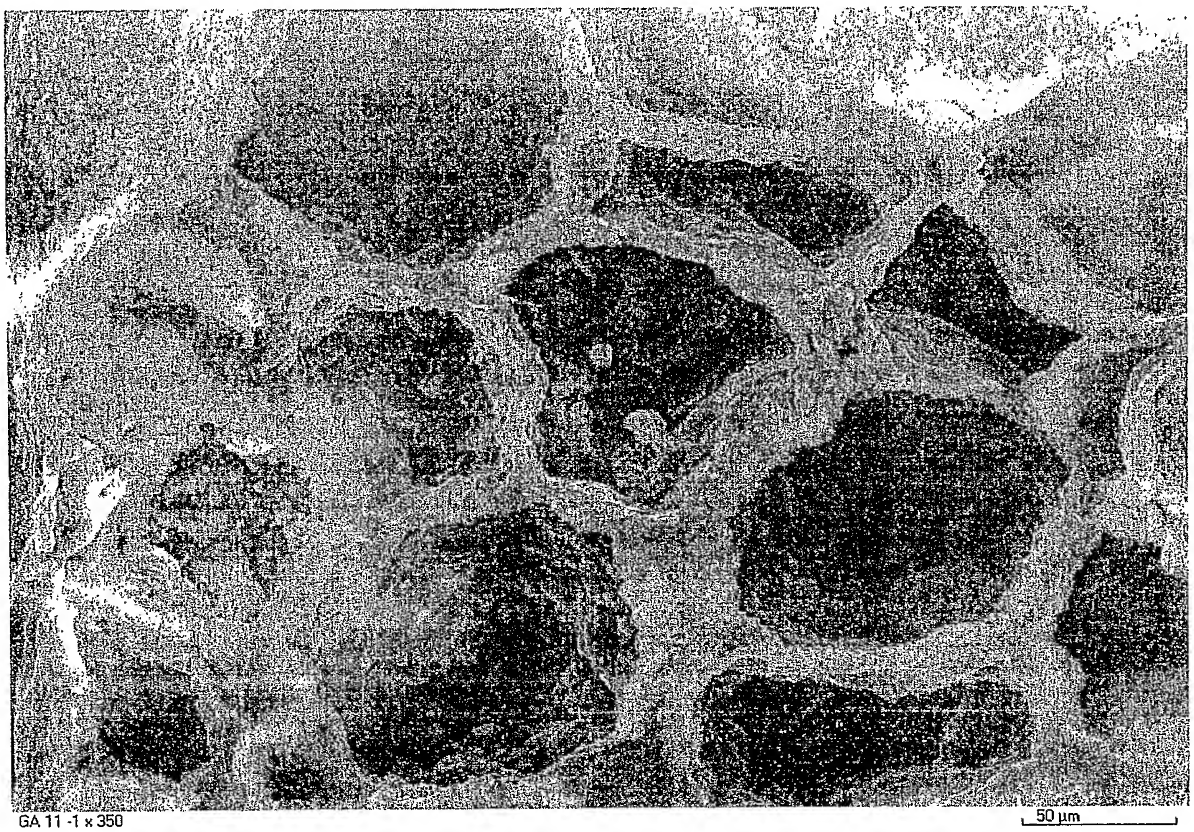


Fig. 1 (d)



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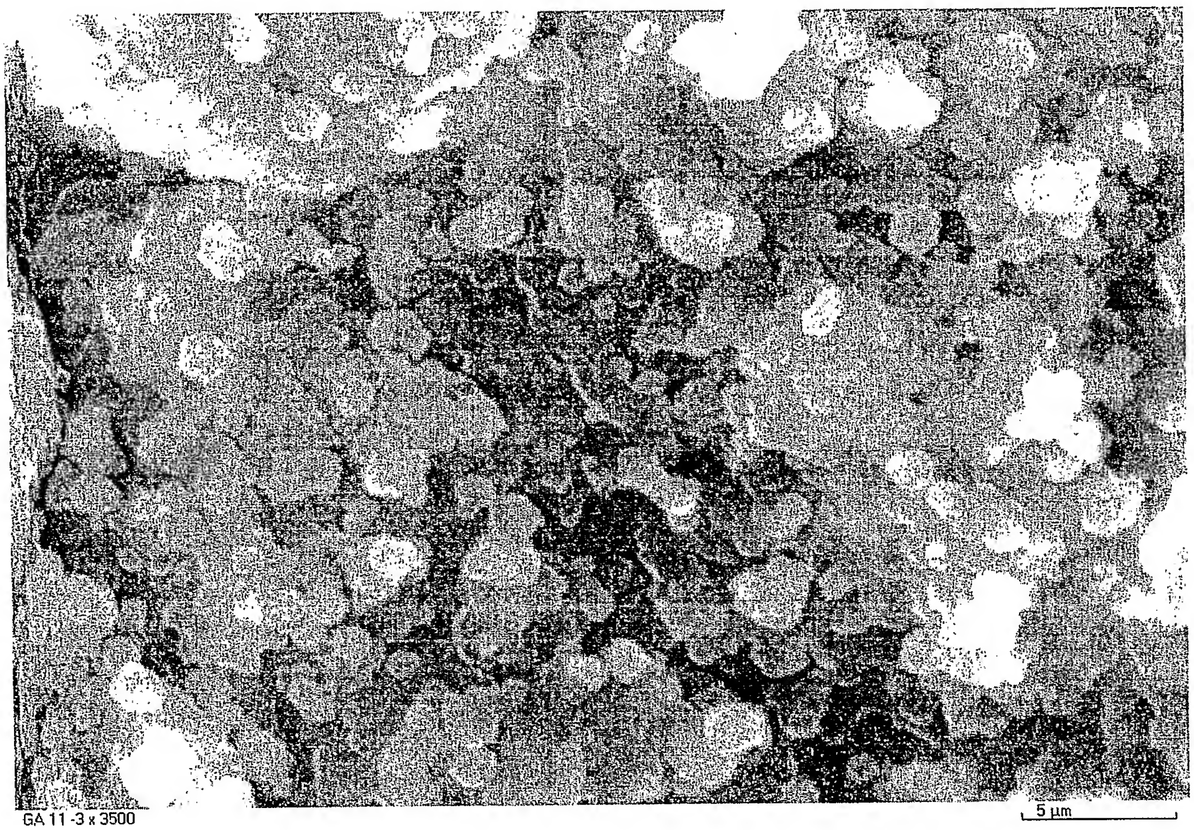
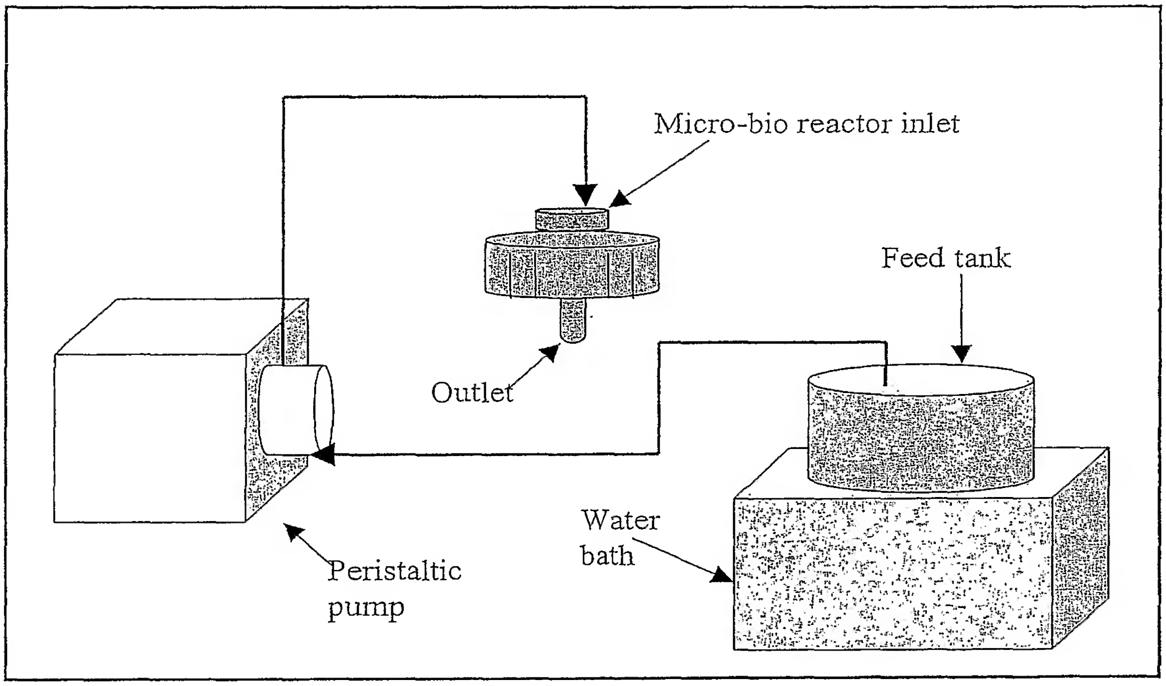


Fig. 2 (b)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Feed tank
Outlet
Water
bath
Peristaltic
pump

Fig. 3 (a)

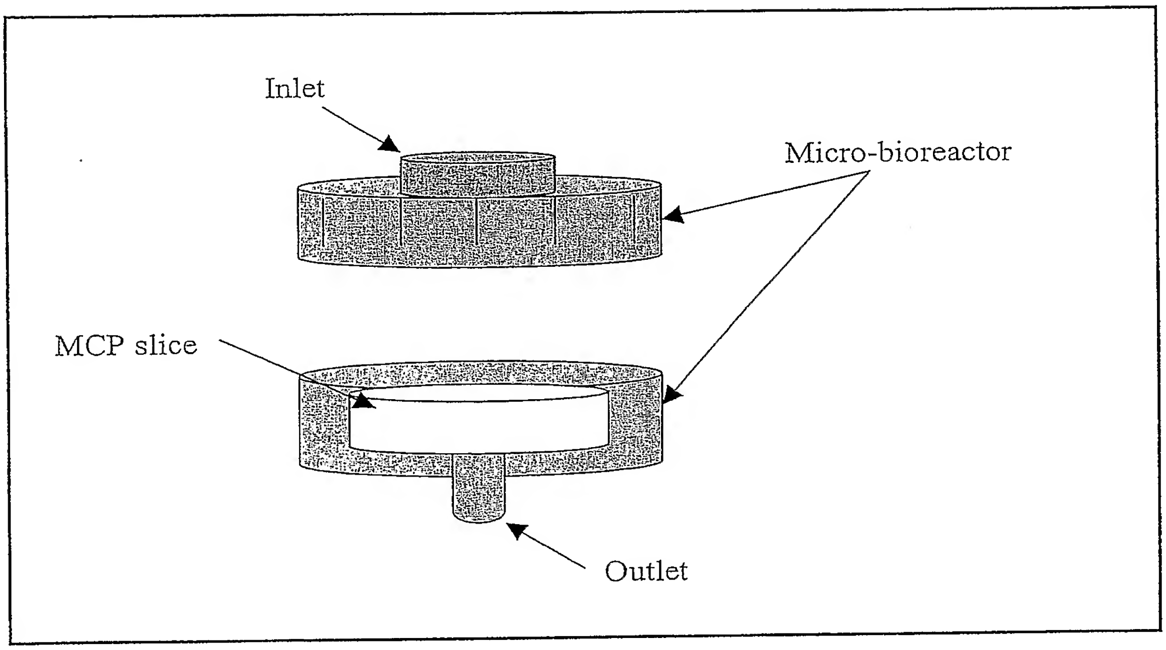
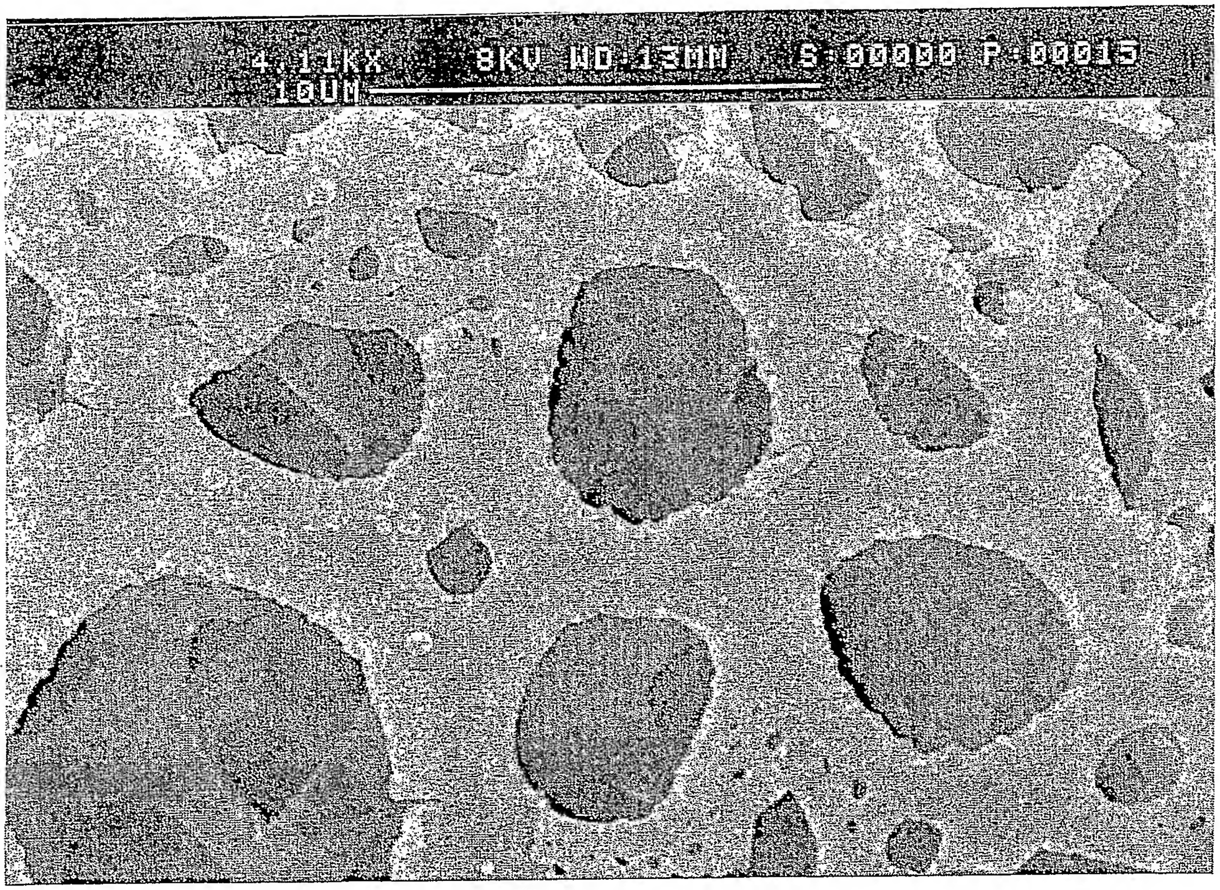


Fig. 3 (b)



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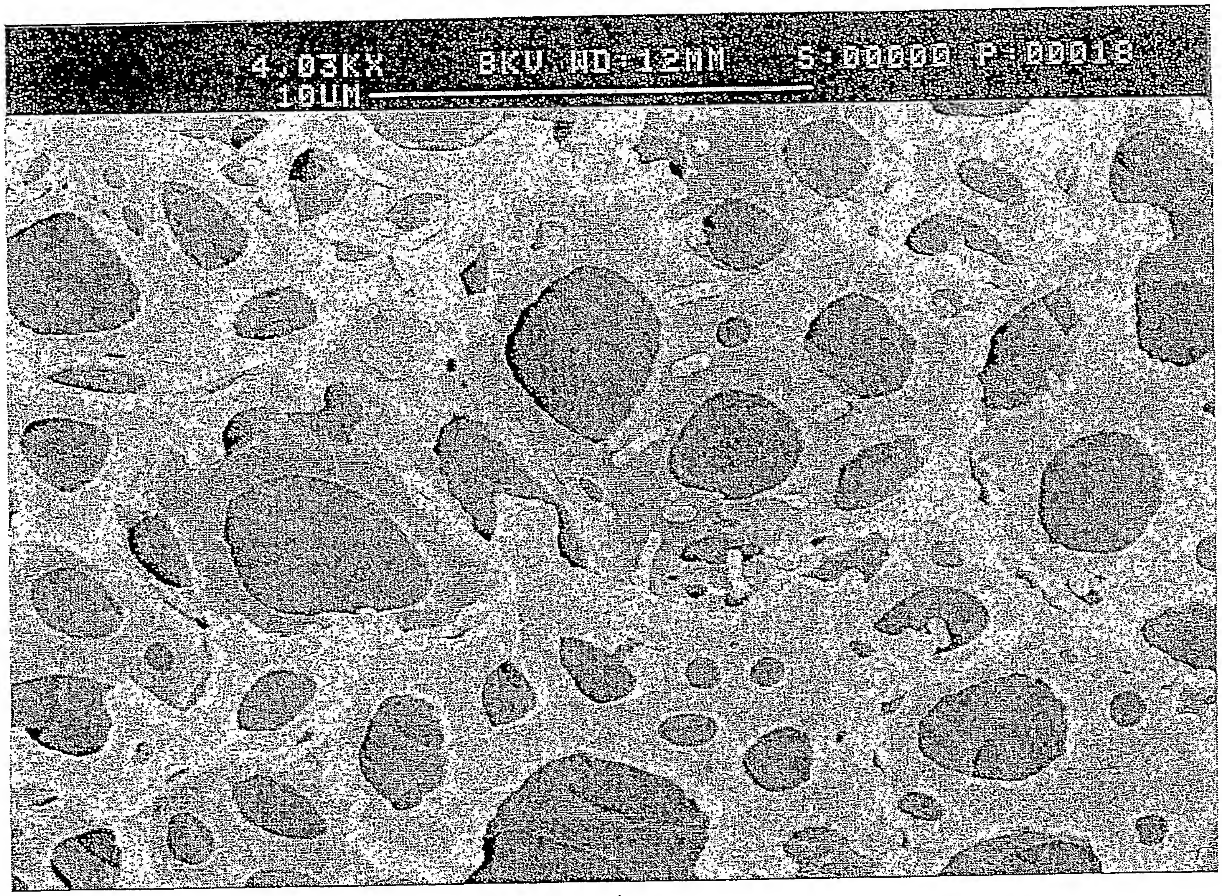


Fig. 4 (b)

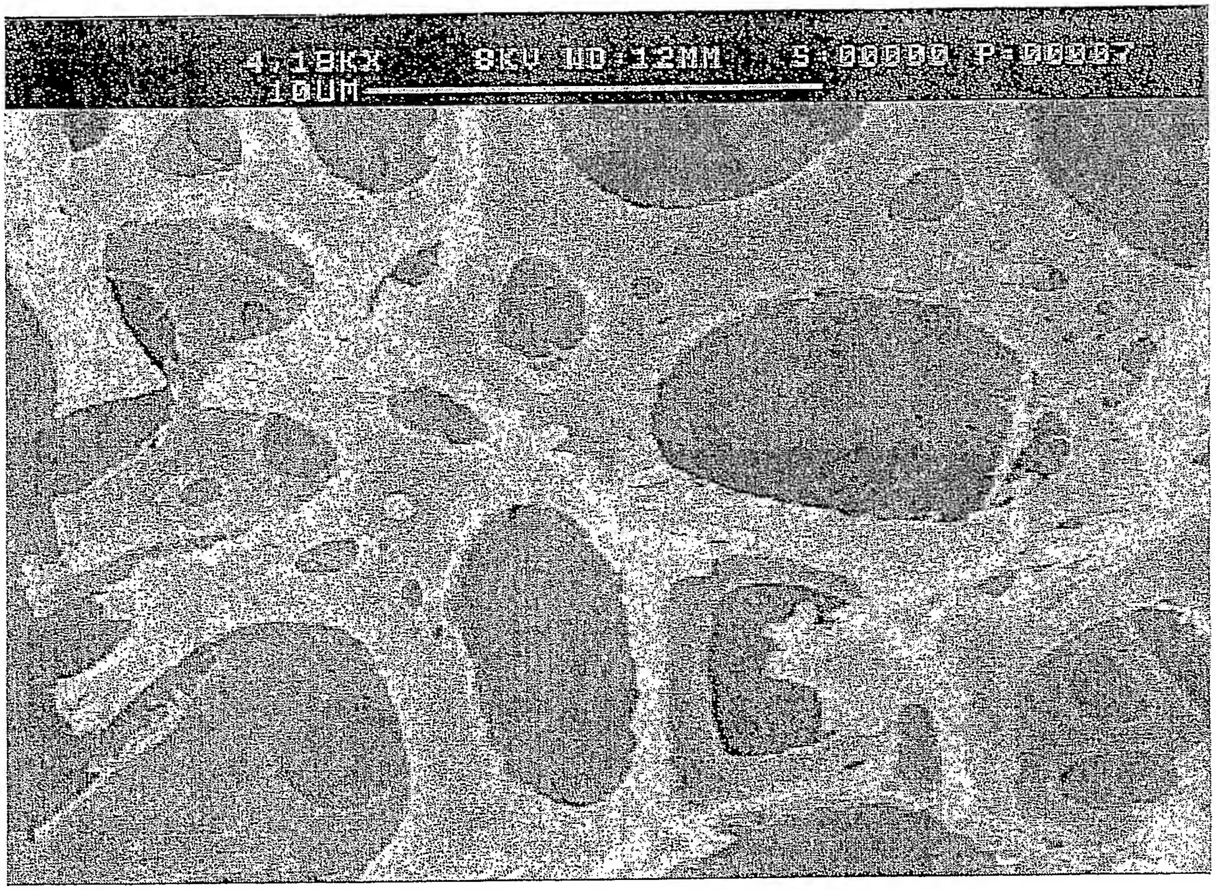


Fig. 4 (c)

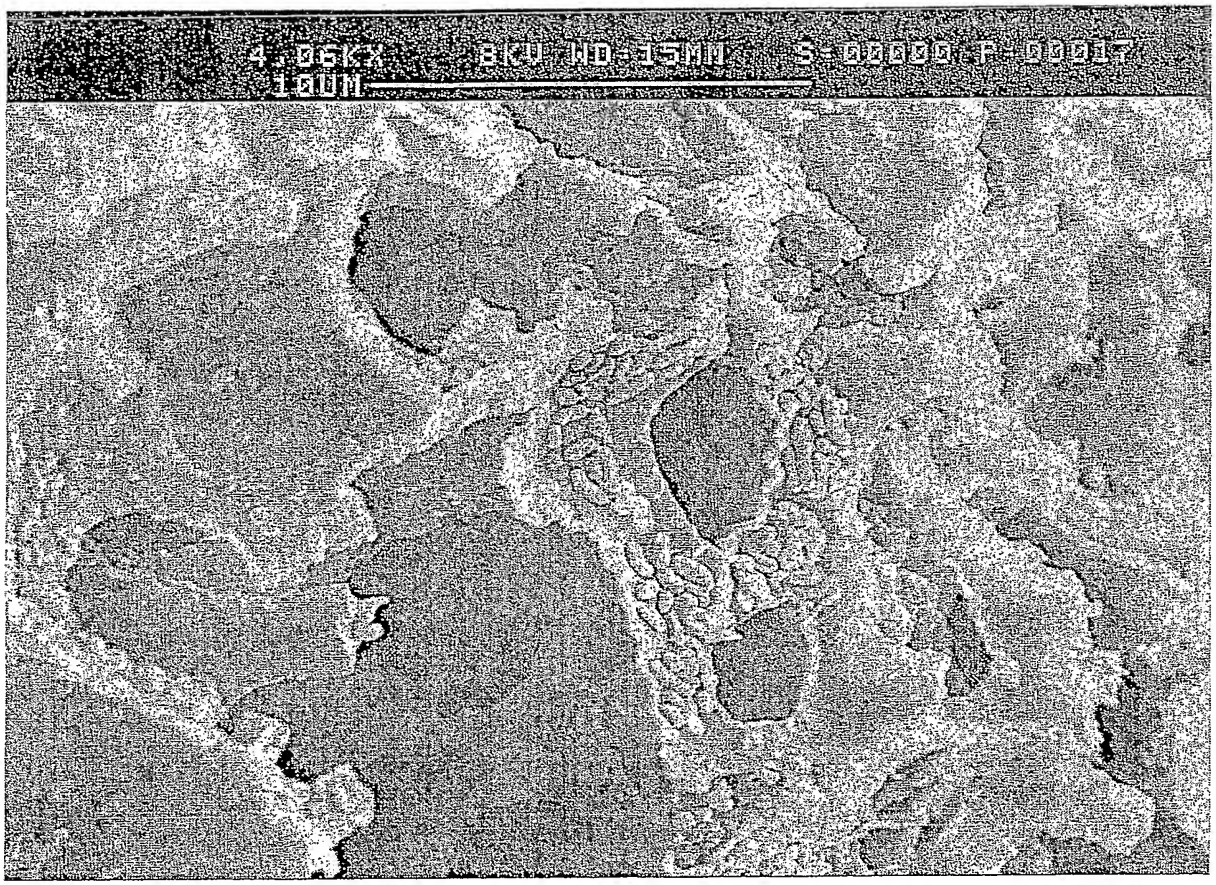
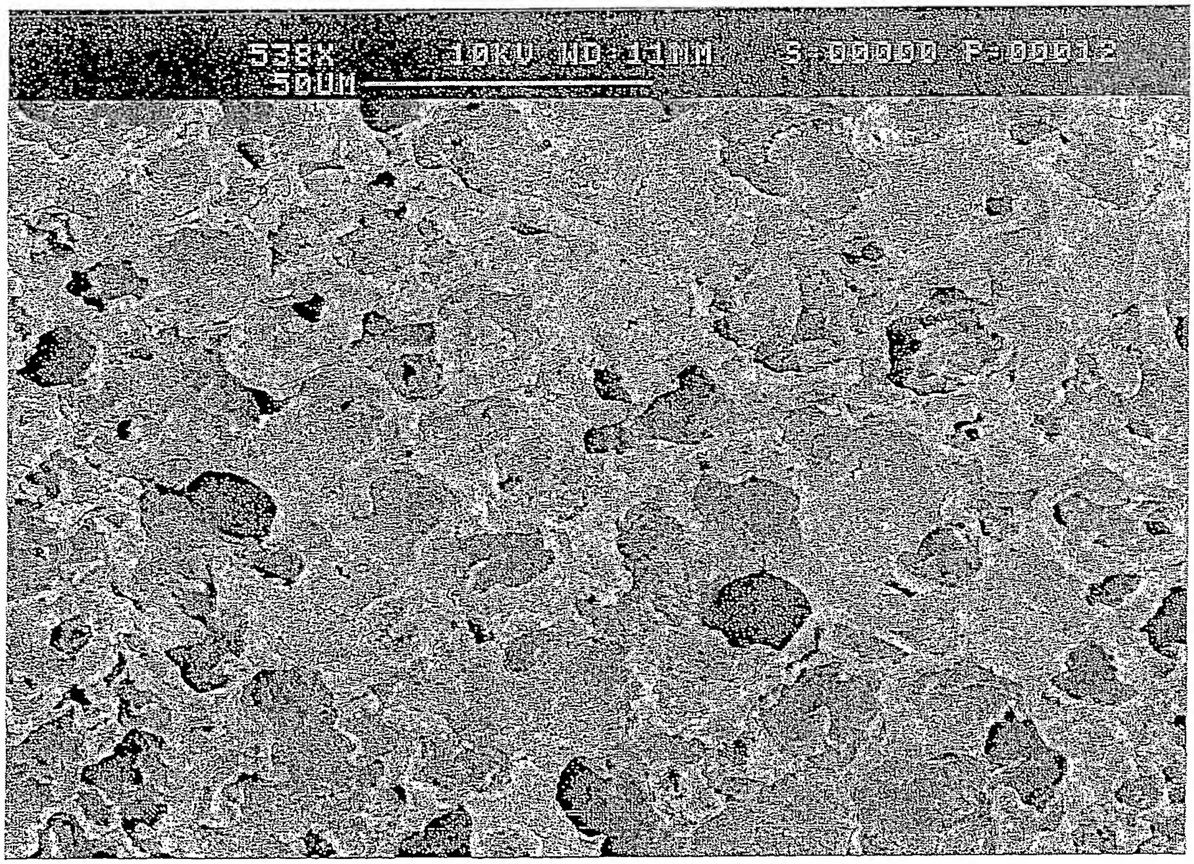


Fig. 5



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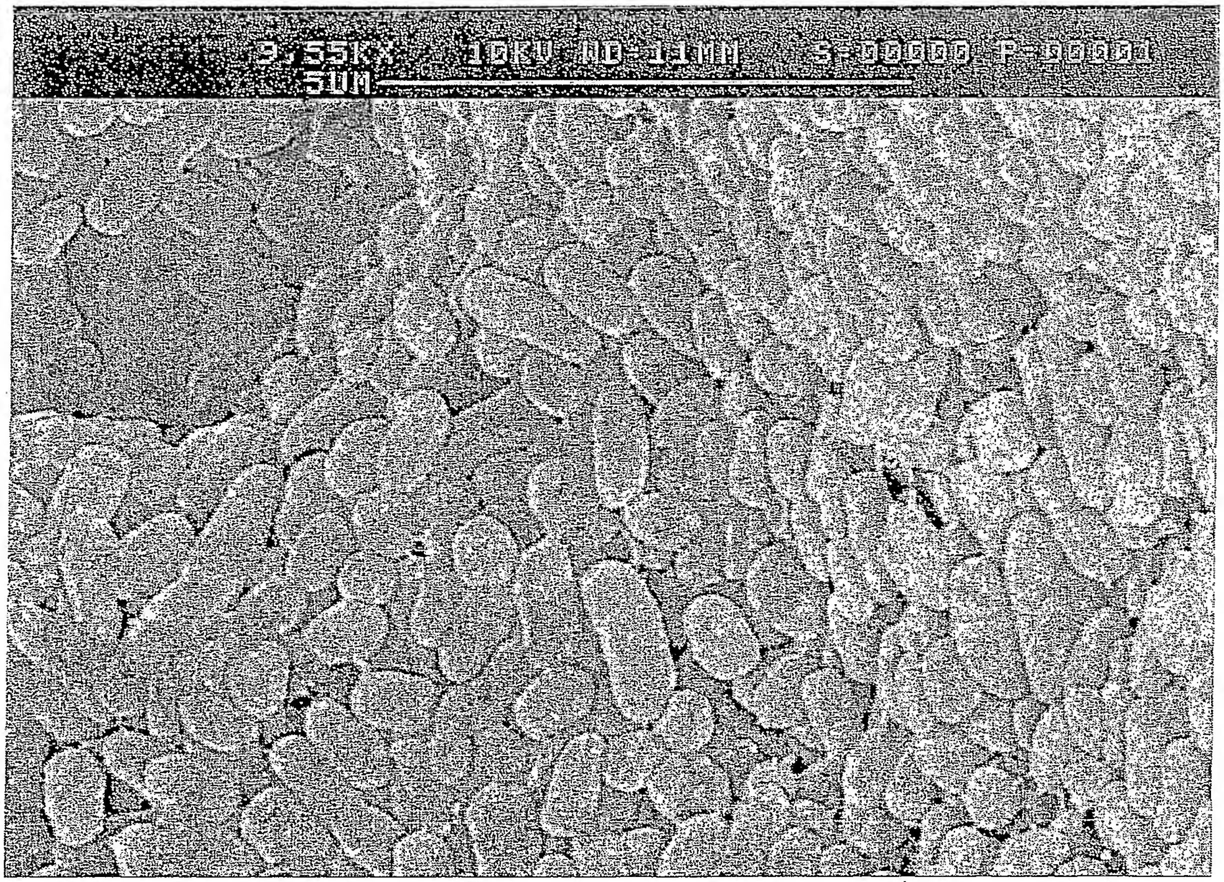


Fig. 6 (b)

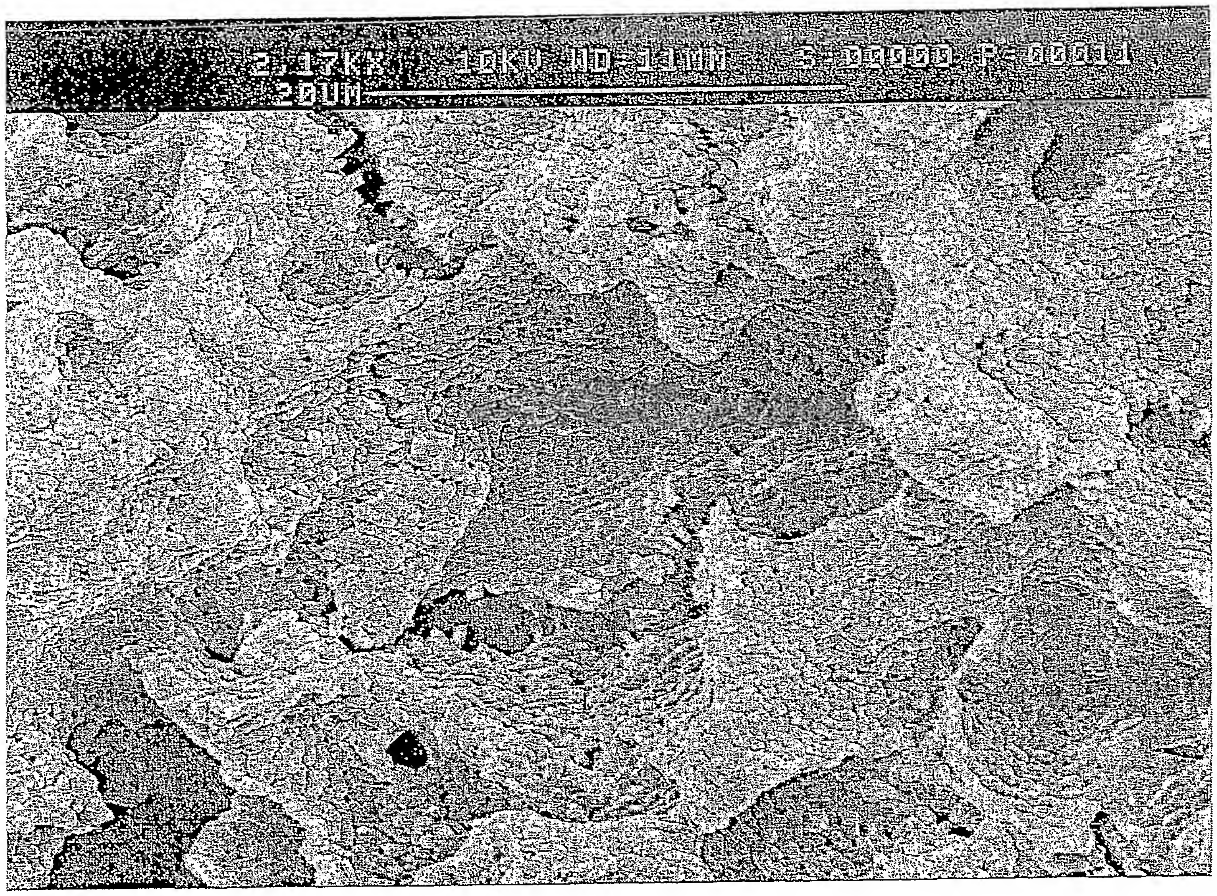


Fig. 6 (c)

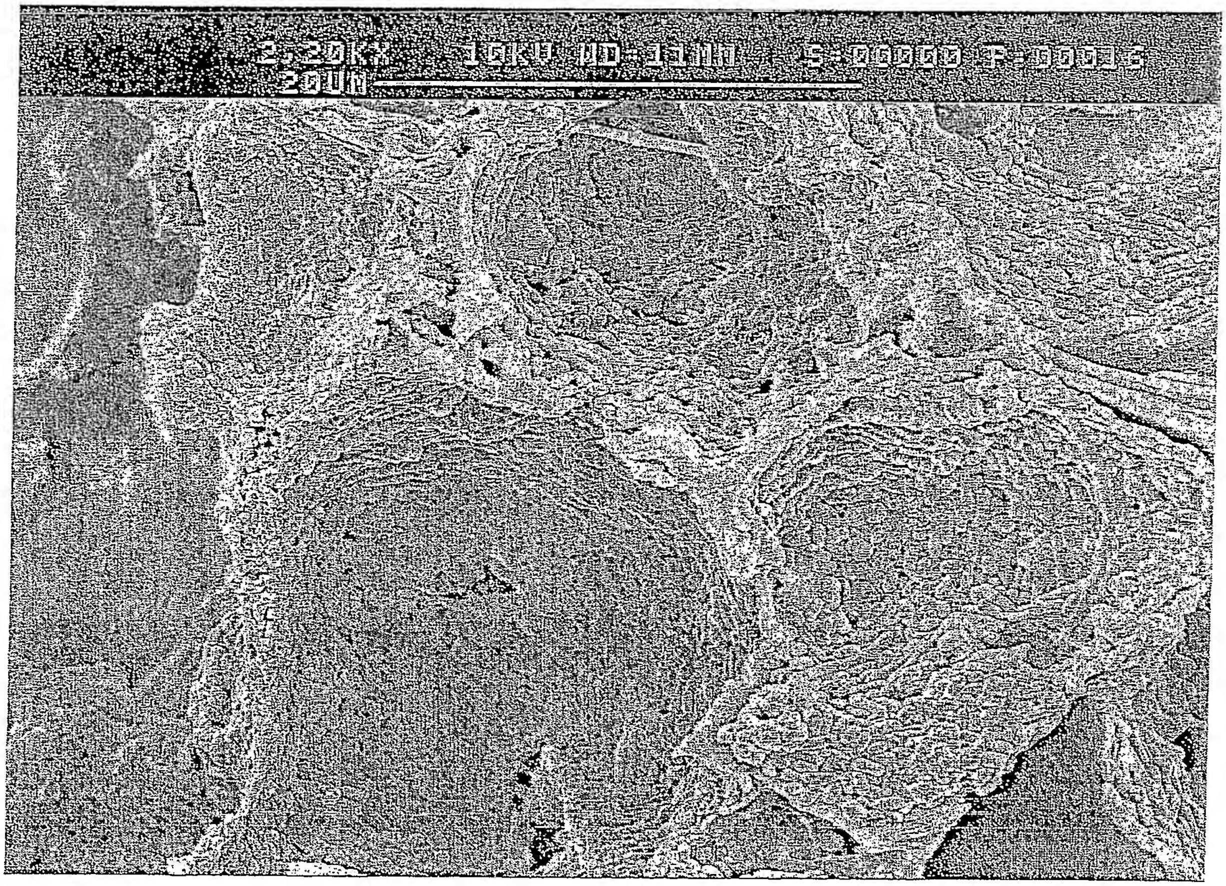


Fig. 6 (d)

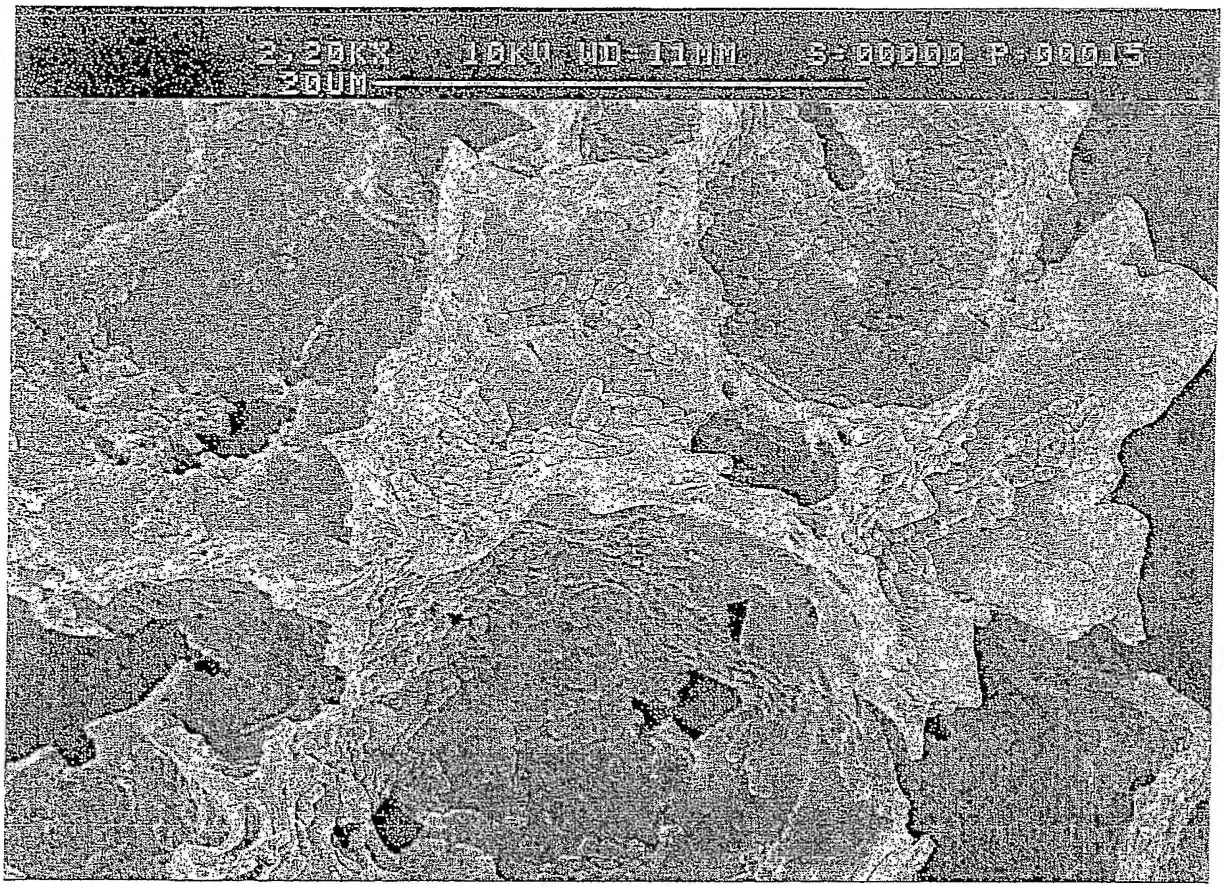
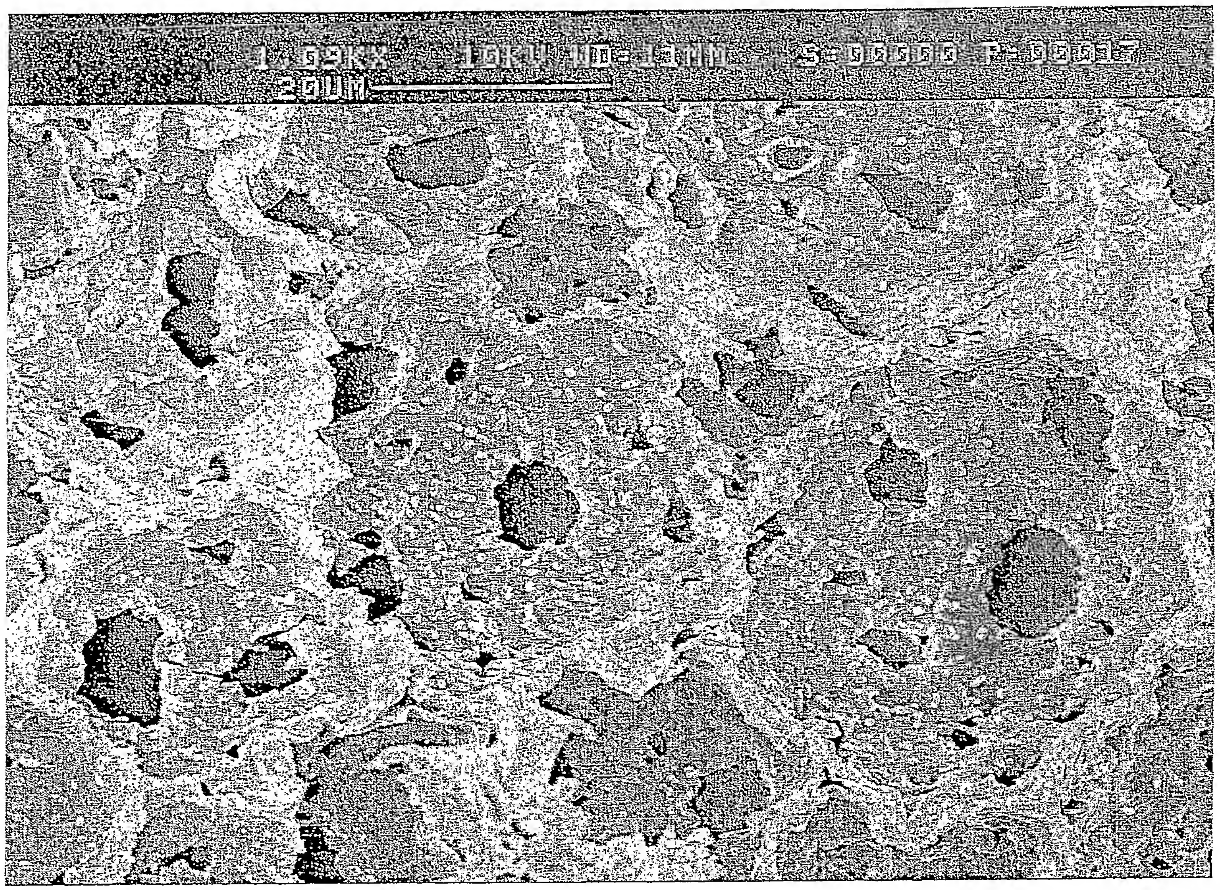


Fig. 6 (e)



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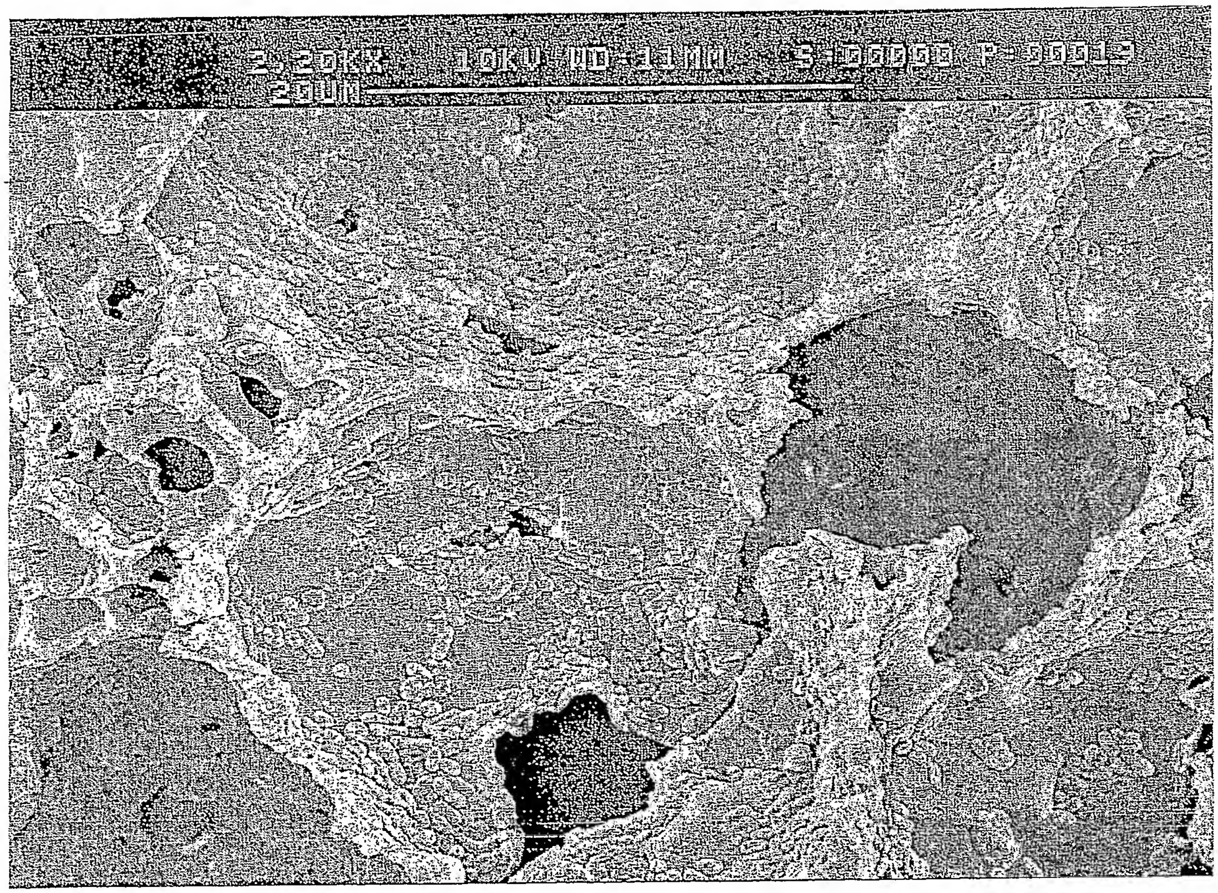


Fig. 6 (g)

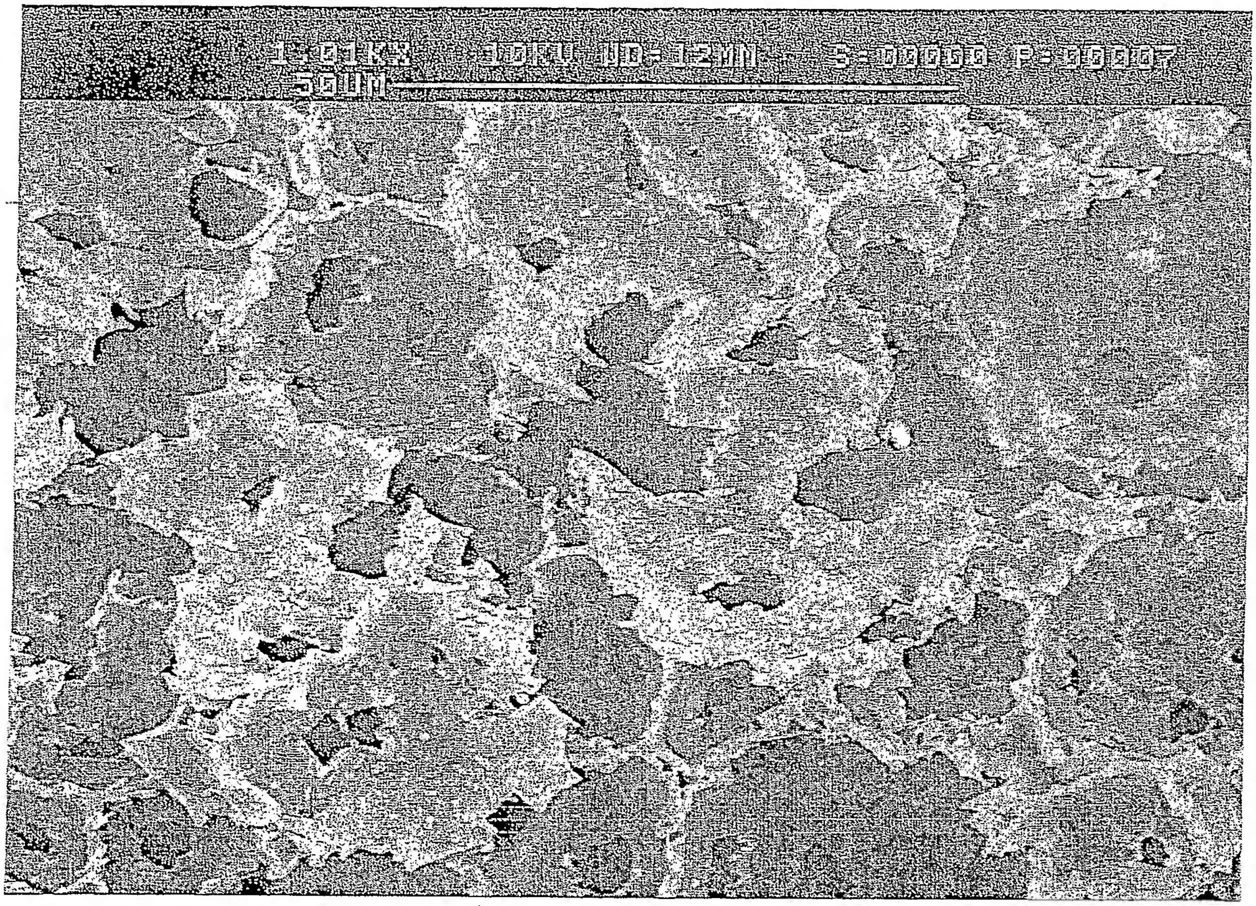
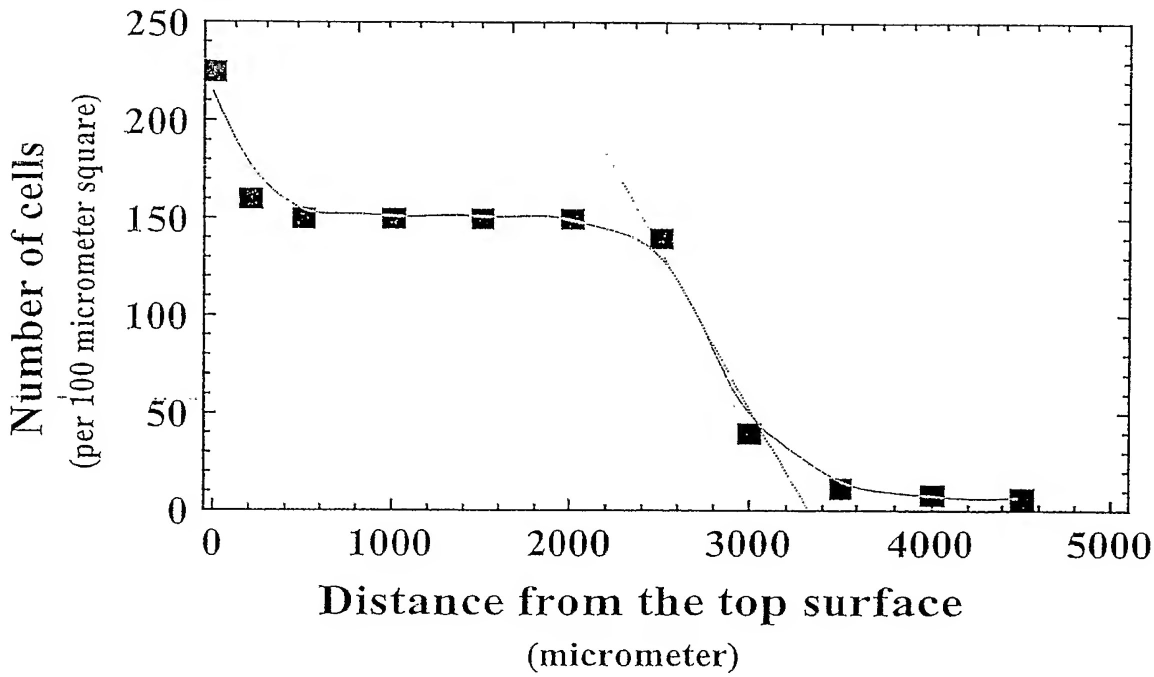


Fig. 6 (h)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200
150
100
50
0
Number of cells
(per 100 micrometer square)
0
1000
2000
3000
4000
5000
Distance from the top surface
(micrometer)

Fig. 7

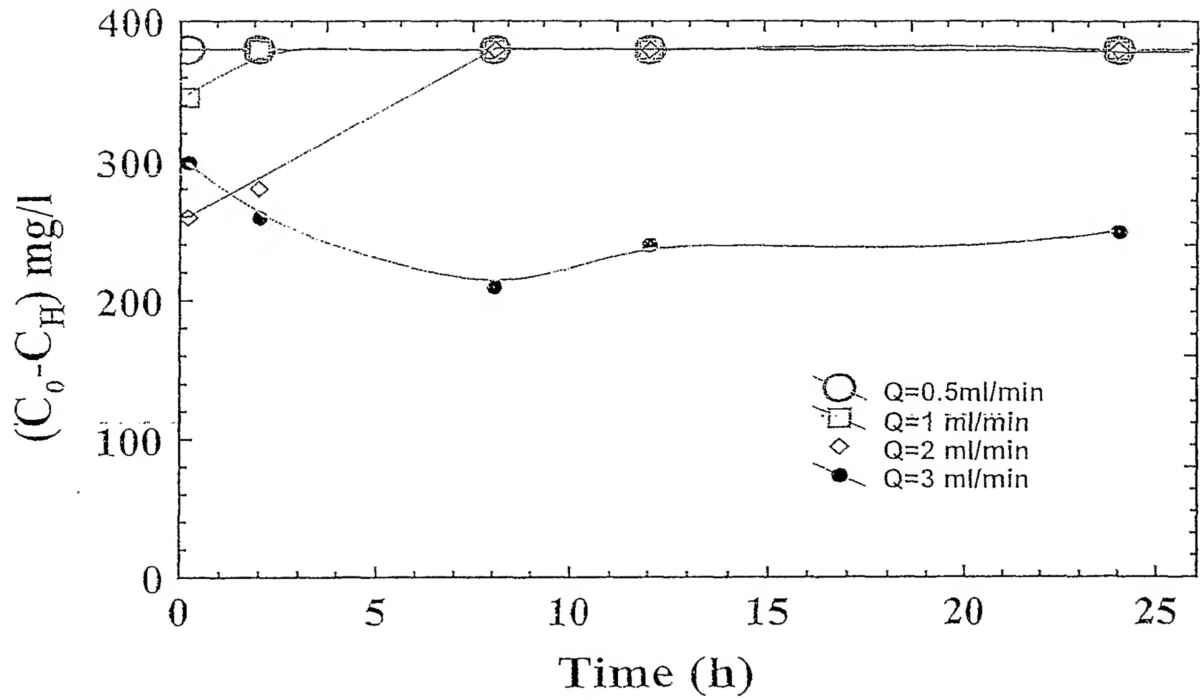

400
300
200
100
0
0
5
10
15
20
25
Time (h)
(C_0-C_H) mg/l
Q=0.5ml/min
Q=1 ml/min
Q=2 ml/min
Q=3 ml/min

Fig. 8

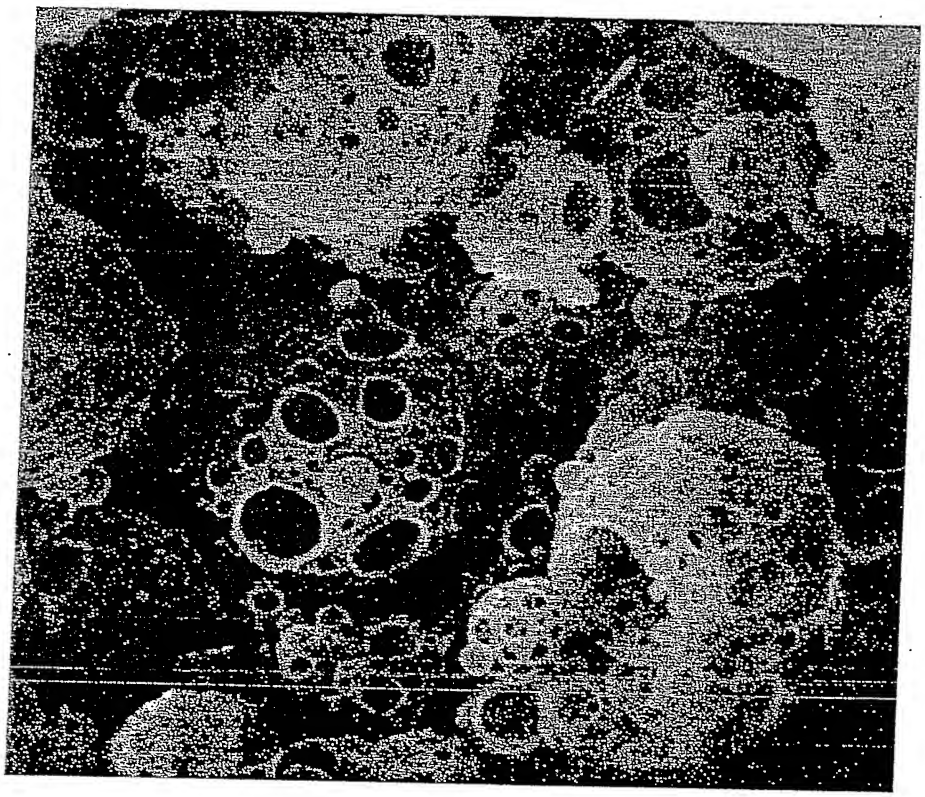


Fig. 9

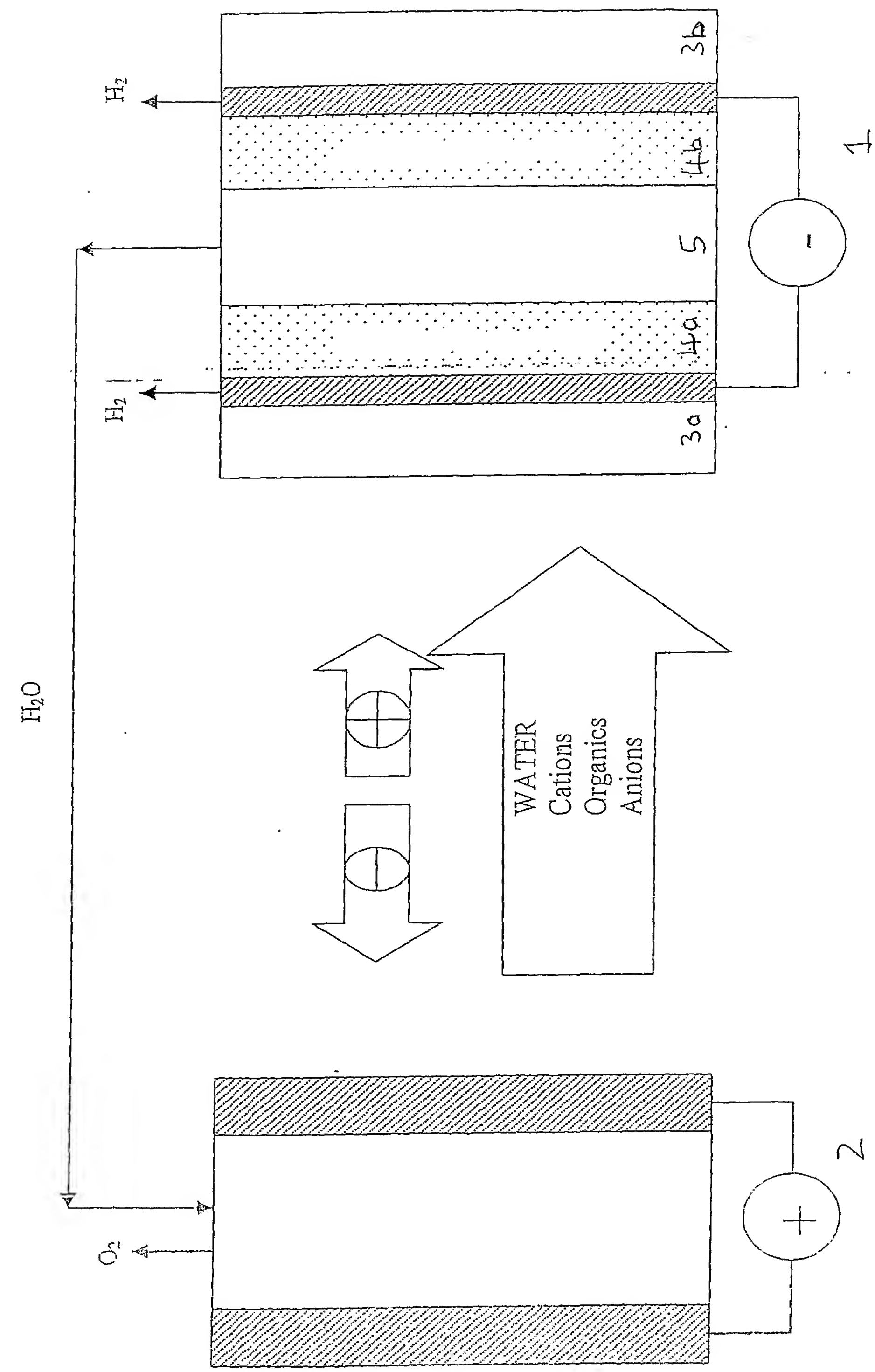


Fig. 10

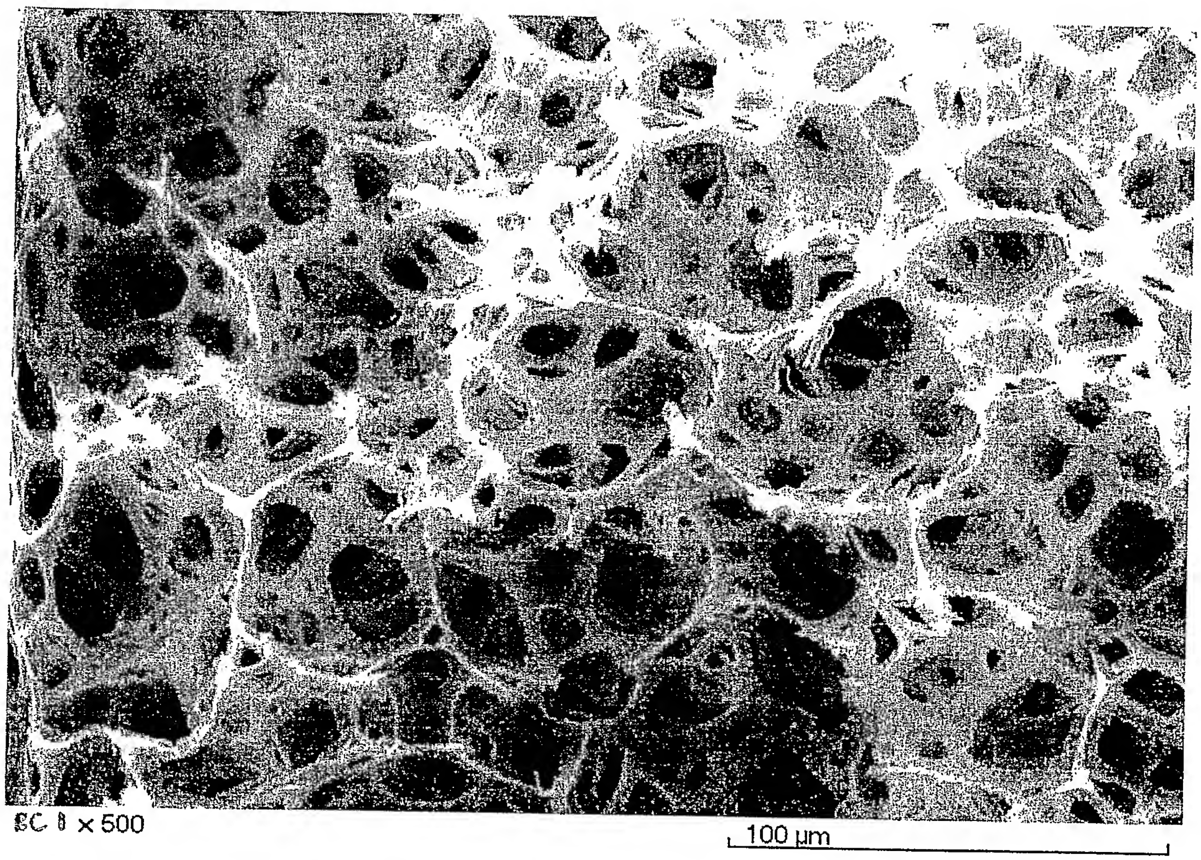


Fig. 11 (a)

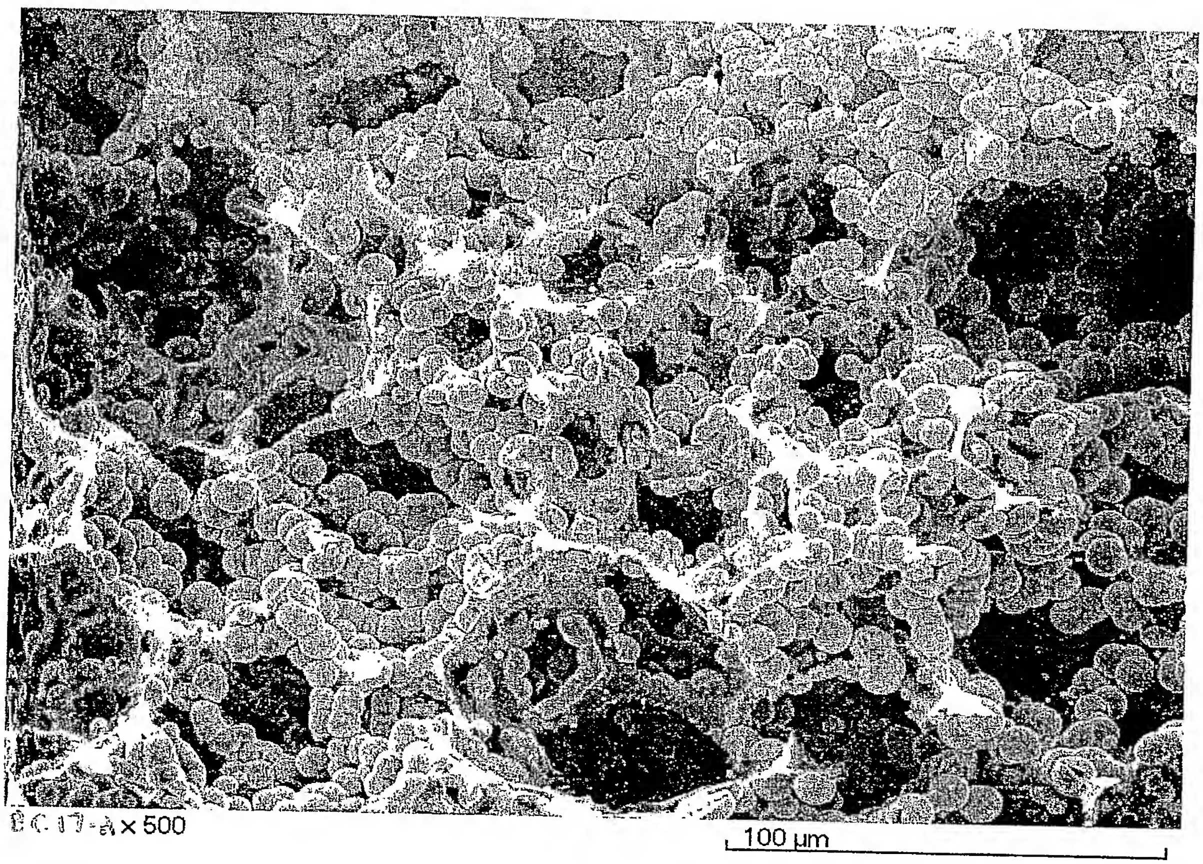


Fig. 11 (b)

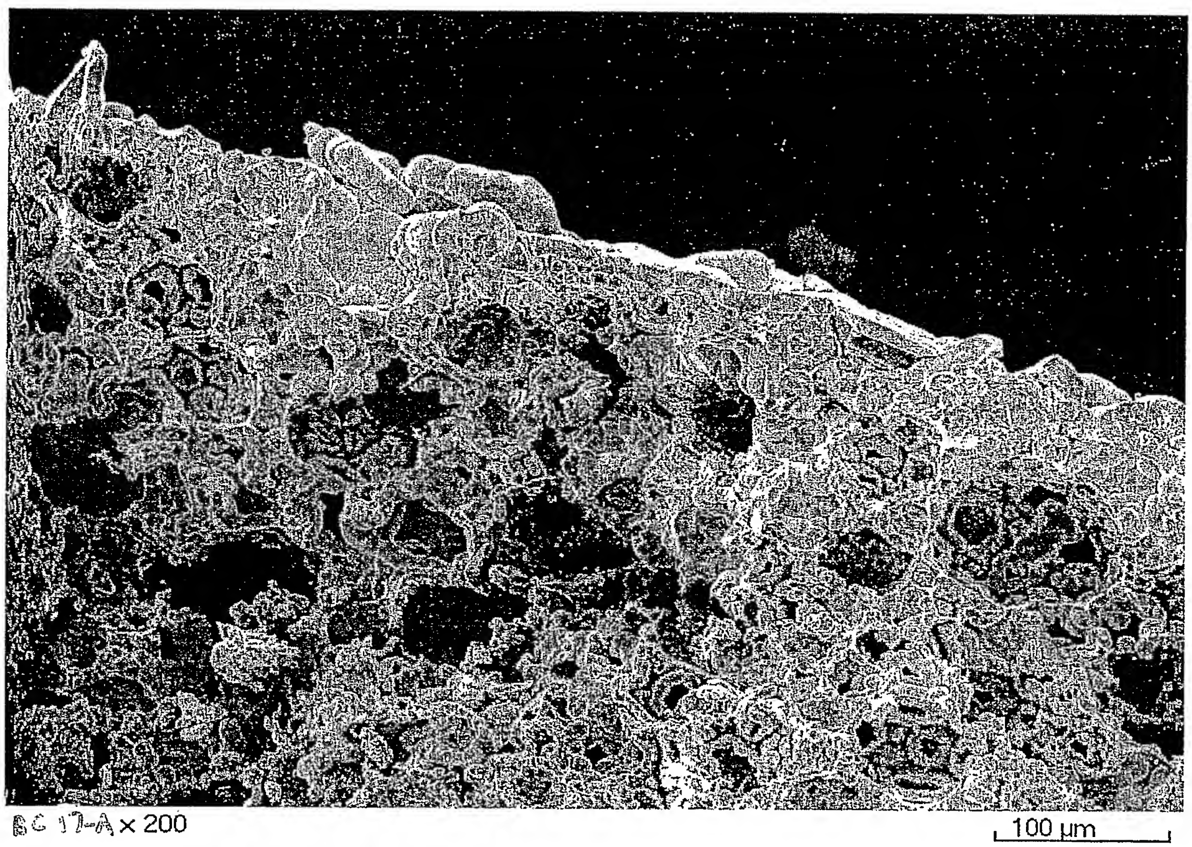


Fig. 11 (c)

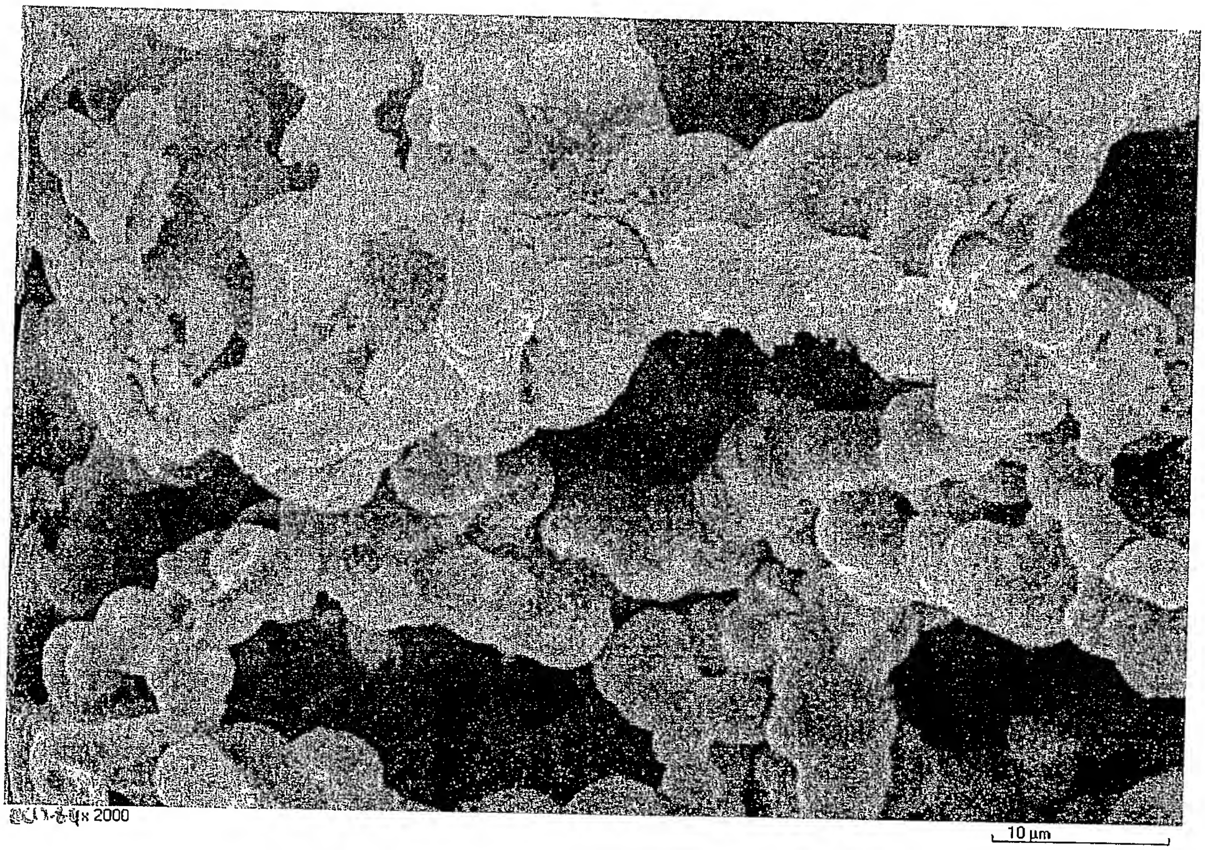


Fig. 11 (d)

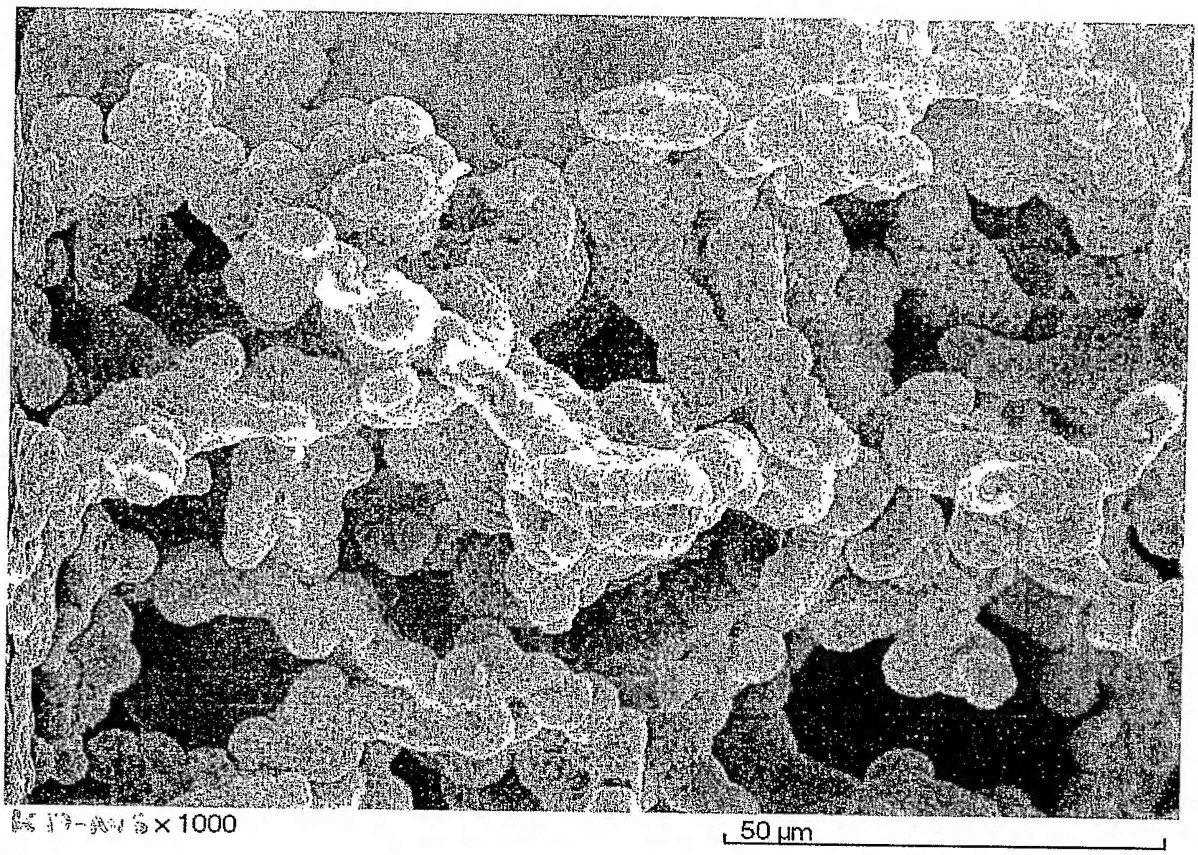


Fig. 11 (e)

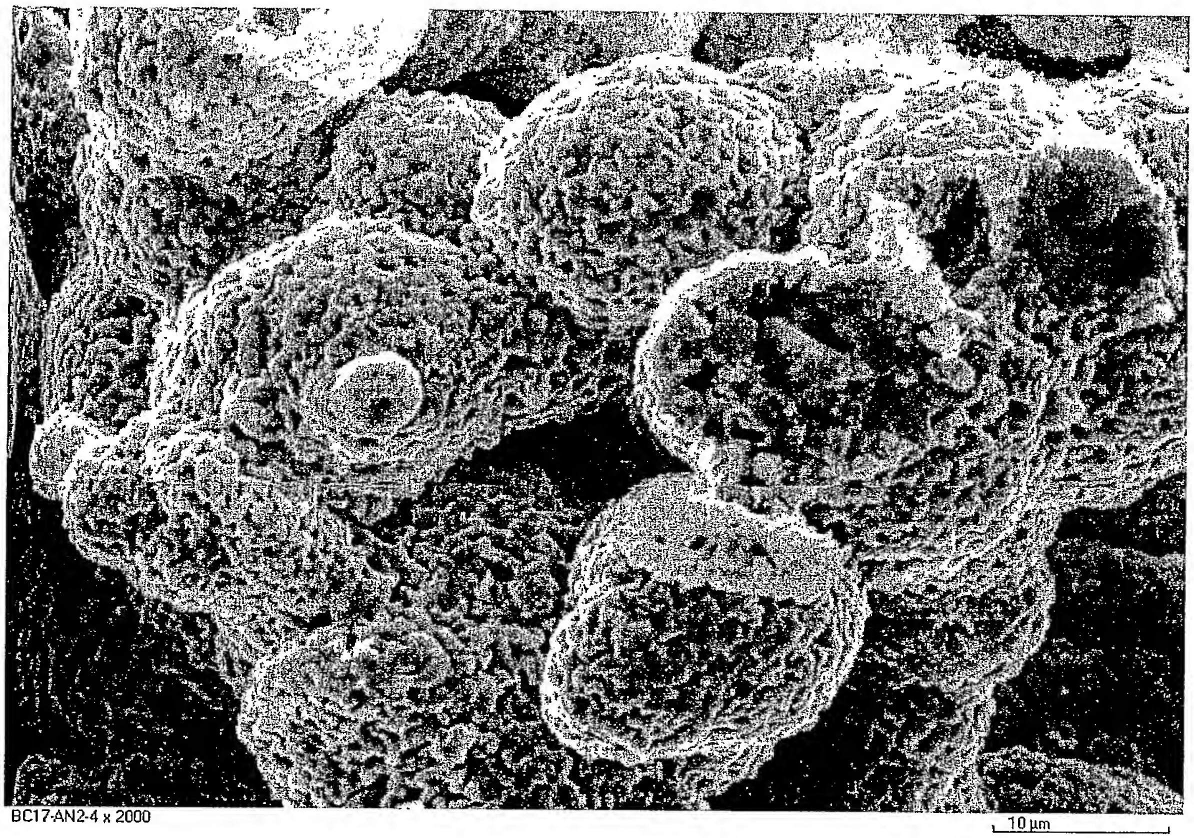


Fig. 11 (f)

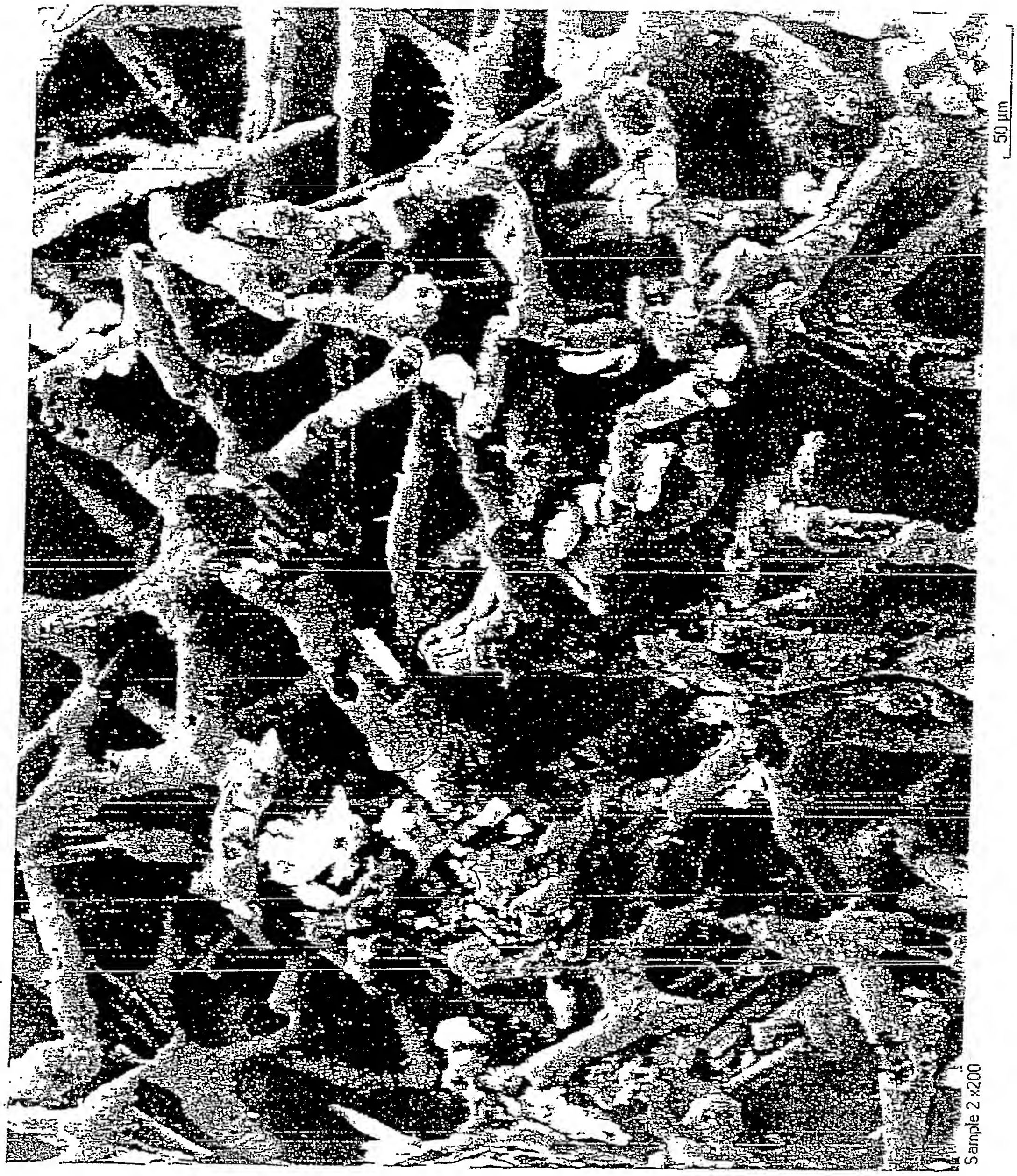


Fig. 11 (g)

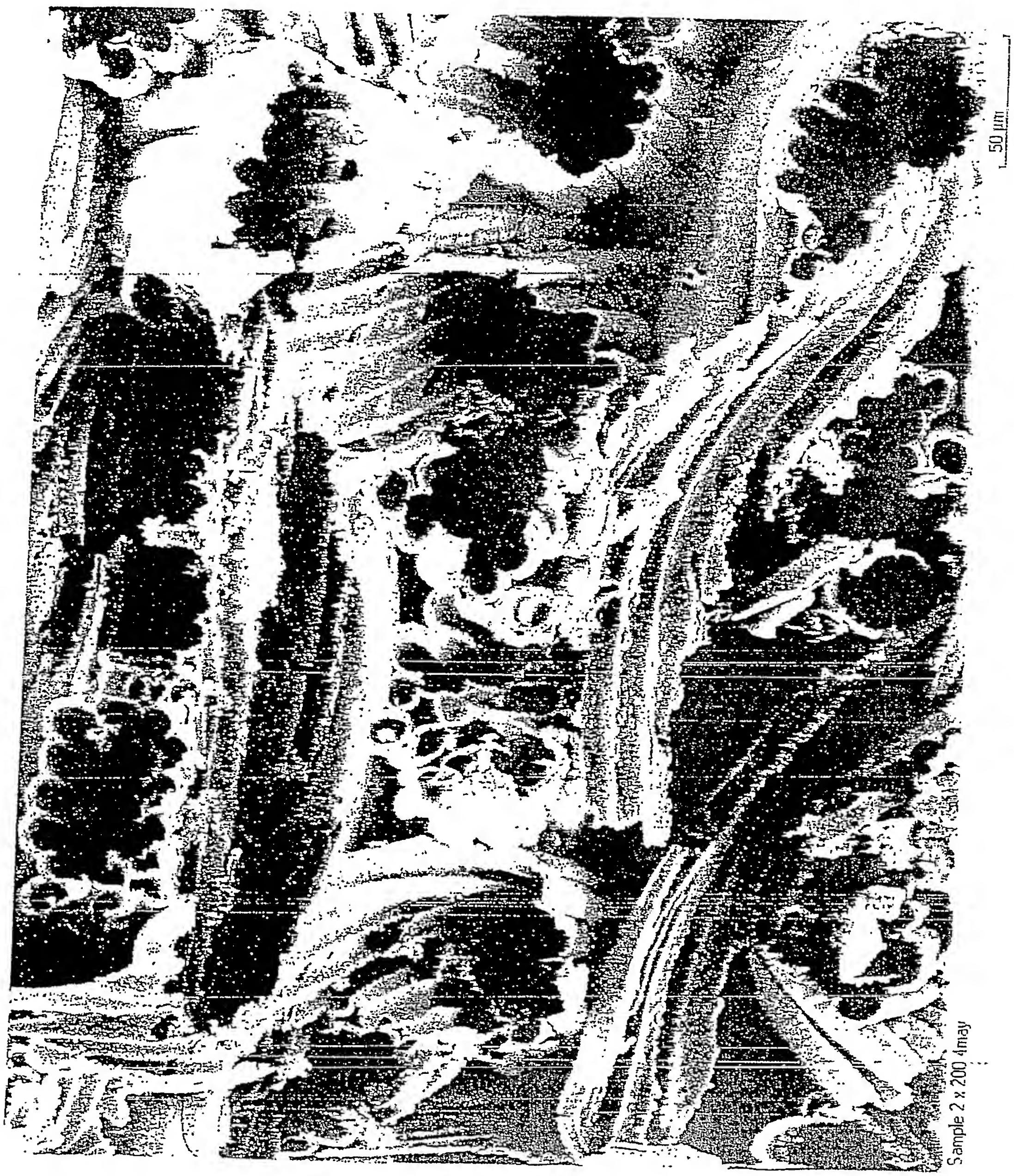


Fig. 11 (h)

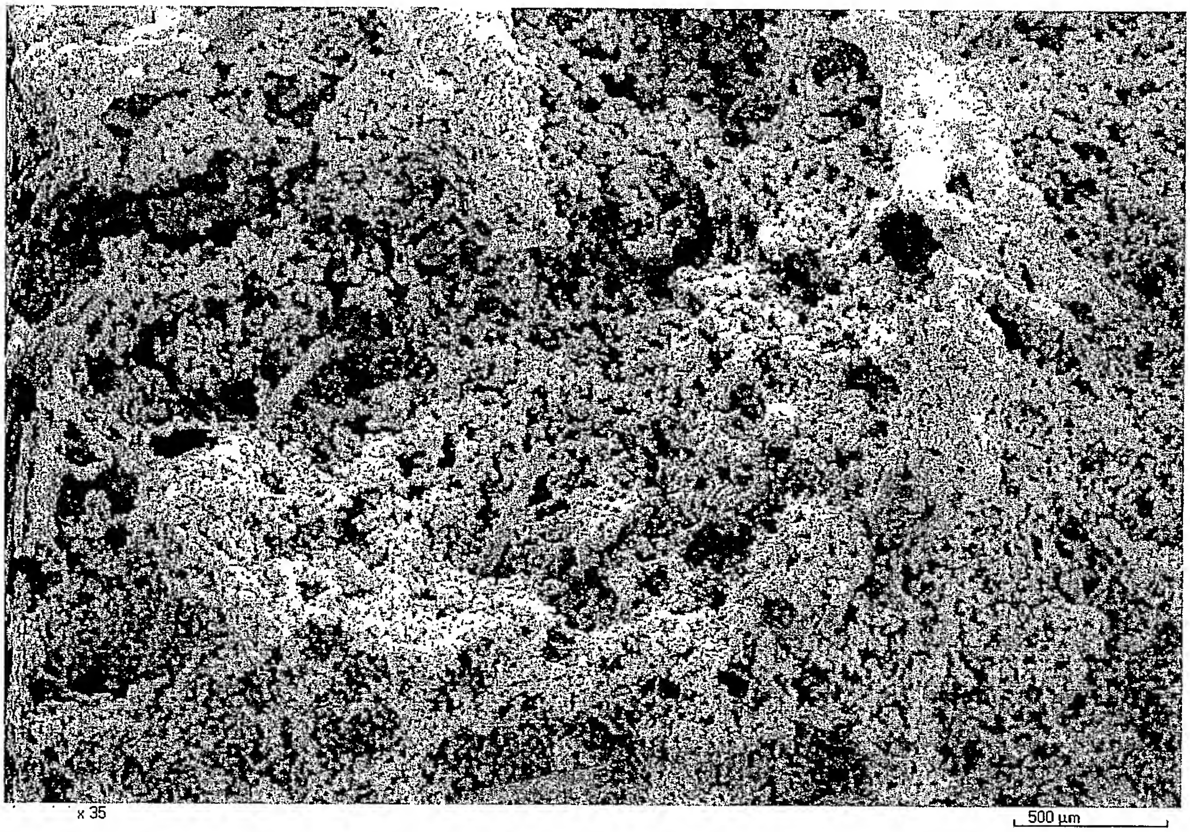


Fig. 11 (i)

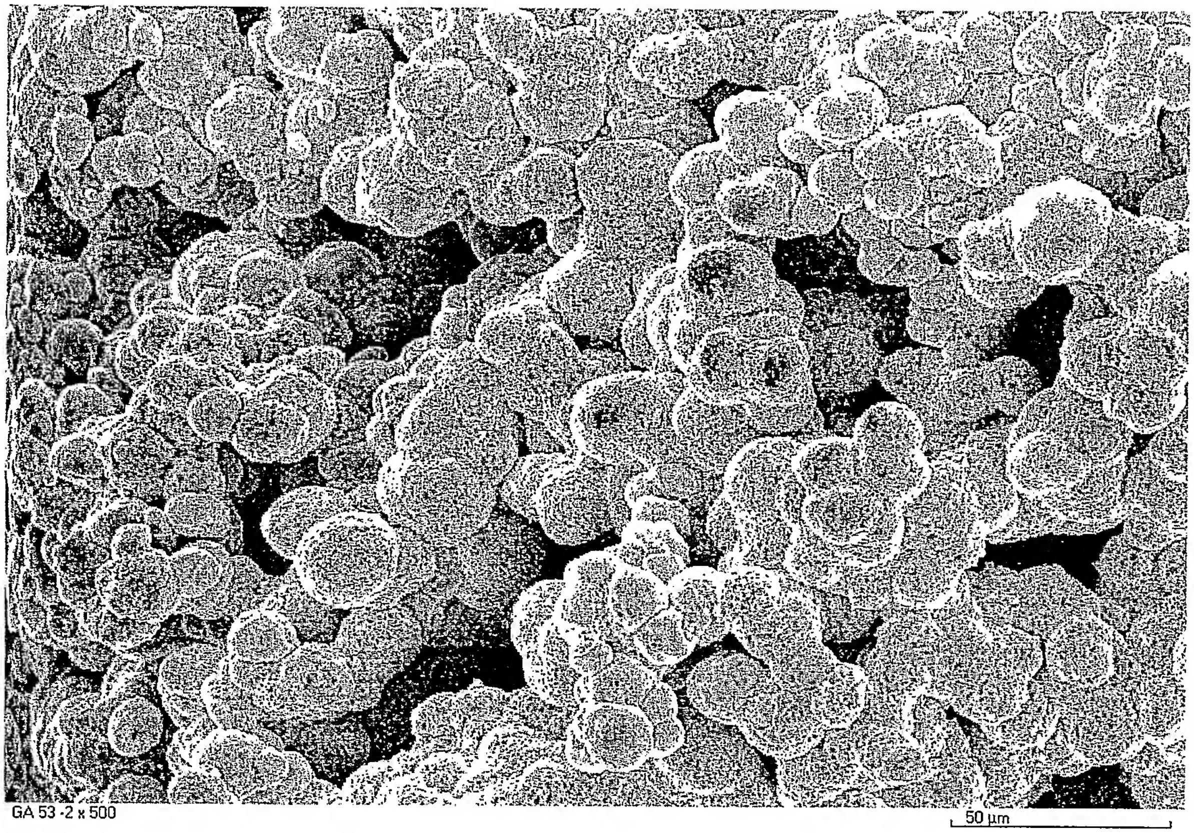
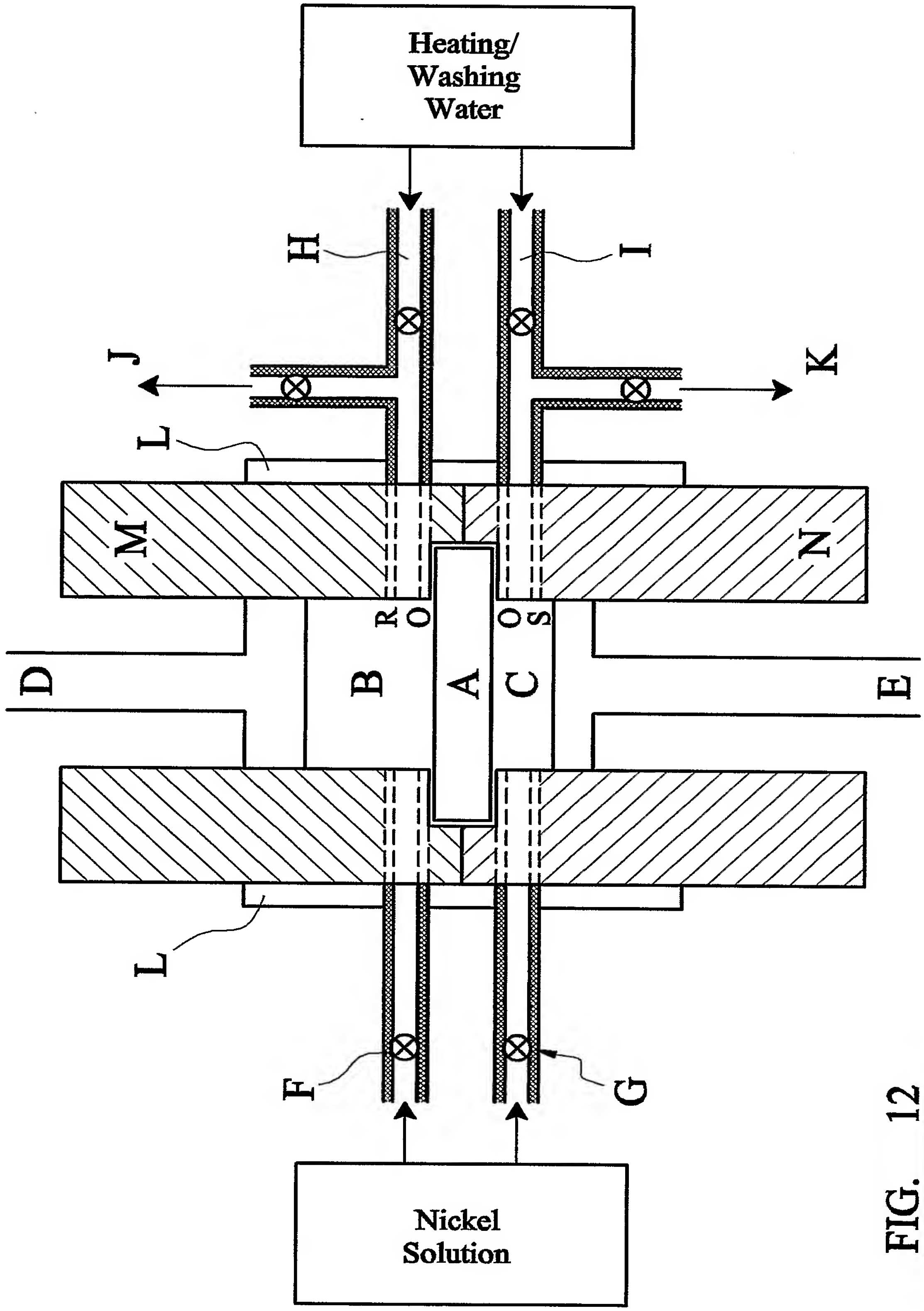


Fig. 11 (j)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Washing
Water
H
I
J
K
L
M
N
R
O
O
S
D
B
A
C
E
L
F
G
Nickel
Solution

FIG. 12

INTERNATIONAL SEARCH REPORT

PCT/GB 03/02975

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C08F2/24 C08F2/22 A61L27/56 A61L27/14 A61L27/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C08F A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, COMPENDEX, IBM-TDB, INSPEC, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00 34454 A (PRICE VICTORIA JANE ;DOWNES SANDRA (GB); AKAY GALIP (GB); UNIV NEW) 15 June 2000 (2000-06-15) cited in the application *whole document*	1-34
X	US 5 071 747 A (HAMMOND KEVIN ET AL) 10 December 1991 (1991-12-10) *whole document*	1-31, 37-45
X	WO 95 33553 A (BIOPORE CORP) 14 December 1995 (1995-12-14) *whole document*	1-26

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 October 2003	10/11/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gold, J

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

PCT/GB 03/02975

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02 10070 A (AKAY GALIP ;UNIV NEWCASTLE (GB); VICKERS JOHN (GB)) 7 February 2002 (2002-02-07) page 4, line 8 - line 9 page 10, line 19 - line 29	16
X	GB 687 201 A (LUCIEN PAUL BASSET) 11 February 1953 (1953-02-11) *whole document*	37-45
X	WO 96 32252 A (GORE & ASS) 17 October 1996 (1996-10-17) *whole document*	37-45
X	DE 42 14 905 A (SILBERKRAFT LEICHTAKKU) 11 November 1993 (1993-11-11) *whole document*	37-45

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/GB 03/02975

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 0034454	A	15-06-2000	AU	1575700	A	26-06-2000
			EP	1183328	A2	06-03-2002
			WO	0034454	A2	15-06-2000
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